



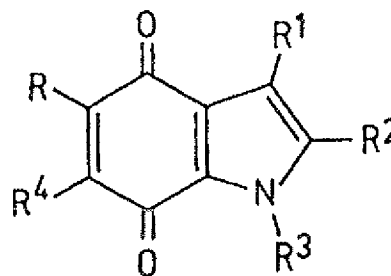
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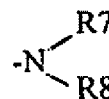
(54) Title: INDOLOQUINONE DERIVATIVES AS BIOREDUCTIVE AGENTS

(57) Abstract

Use of a compound of general formula (I) or salt thereof wherein R and R⁴ are independently selected from hydrogen, halogen and C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl, C₁₋₆ alkoxy, phenoxy, C₁₋₆ alkylthio, phenylthio, primary and secondary amino or hydroxy groups and R³ is hydrogen, hydroxy, a C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl or C₁₋₆ alkoxy or haloalkoxy group is provided for the manufacture of a medicament for the treatment of neoplasms, particularly solid cancerous tumours characterised in that R¹ is selected from a C₁₋₆ alkyl or haloalkyl group, -CO₂R⁵ where R⁵ is hydrogen or a C₁₋₆ alkyl or haloalkyl group, or a group -CH₂-X where X is selected from groups of formula -S-R⁶, -O-R⁶, and (a) where R⁶ is a hydrogen or a leaving group, the acid HR⁶ of which has a pK_a of 10 or less and R⁷ and R⁸ are the same or different and are selected from C₁₋₆ alkyl or haloalkyl or together with the interjacent nitrogen form a heterocyclic ring of 5 to 7 atoms optionally substituted by C₁₋₄ alkyl or haloalkyl and R² is selected from hydrogen, C₁₋₄ alkyl and haloalkyl or groups -(CH₂)_nCHR⁹R¹⁰ of more than four carbon atoms where n is an integer of 0 to 2 and R⁹ and R¹⁰ are independently selected from a C₁₋₄ alkyl or haloalkyl group, or R⁹ and R¹⁰ together with the interjacent carbon atom form a C₃₋₇ cycloalkyl or cycloalkenyl ring optionally substituted with one or more C₁₋₄ alkyl or haloalkyl, or C₂₋₄ alkenyl or haloalkenyl groups.



(I)



(a)

or R⁹ and R¹⁰ together with the interjacent carbon atom form a C₃₋₇ cycloalkyl or cycloalkenyl ring optionally substituted with one or more C₁₋₄ alkyl or haloalkyl, or C₂₋₄ alkenyl or haloalkenyl groups.

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INDOLOQUINONE DERIVATIVES AS BIOREDUCTIVE AGENTS

The present invention relates to the use of a group of indoloquinones as therapeutic agents, particularly as bioreductively activated anti-tumour agents, to novel members of this group and compositions containing them and to methods of using these to treat tumours.

5 Fused cyclopropamitosenes and closely related indoloquinones have recently been evaluated as novel bioreductive anticancer agents (references 1-3 below). These compounds were originally designed as analogues of the archetypal quinone bioreductive alkylating agent mitomycin C (Figure 1 herein; MMC, compound 1) having much reduced electrophilicity at C-1 due to the inertness of the 1,2-cyclopropane compared to the aziridine
10 in MMC. Certain indoloquinones (e.g. Figure 1; compound 2) in this series have been found to be highly potent cytotoxins as compared with MMC and in some cases have substantially higher hypoxic cytotoxicity ratios (HCR) (see references 2-3). The cyclopropamitosenes have been found to be more rapidly reduced by DT-diaphorase, an important activator of mitosenes, compared to MMC, but this could not explain fully the
15 higher potency of the compounds compared to the current lead clinical agent of this type EO9 (3), which is reduced two orders of magnitude more rapidly than compound 2 by DT-diaphorase (see WO 87/06227 \equiv US 5097257).

The presence of particular groups as the 5-substituent, in particular aziridines and substituted aziridines, and as the leaving group on the 3-methylene substituent was
20 therefore identified as an important feature, both in terms of oxic and hypoxic potency and rate of reduction, i.e. ability to act as substrates for reductase enzymes. This does not vary greatly among 5-aziridine and 5-methoxy derivatives (see references 2 and 6 below), but it should be noted that the relative rates of reduction of this type of compound by one-electron reductases, which will be important under hypoxic conditions, is unknown,
25 and there is very little data on one electron reduction potentials of mitosenes.

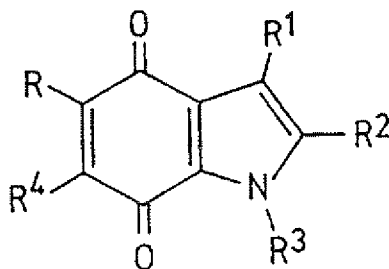
Ionic ring-opening of the fused cyclopropane is considered unlikely but radical ring opening of the cyclopropane to give a reactive H-atom abstractor has been suggested as a possible explanation for hypoxic potency in particular. There has however been no direct evidence for this mechanism.

The present inventors have now designed and synthesized analogues of compound 2 wherein *inter alia* (i) cyclopropane rings are included not fused to the indoloquinone ring system, (ii) lower alkyl eg. isopropyl analogues of these that are structurally very closely related to 2 but unable to undergo radical ring-opening reactions and (iii) analogues
5 containing no substituent, ie. where R^2 is hydrogen. These derivatives have been designed as closer analogues of EO9 with the aim of targeting and exploiting DT-diaphorase activity. Particularly desirable is the optimization of hypoxic-cytotoxicity by such modifications and this novel series of compounds offers many compounds of relatively high HCR.

Some of the compounds used by the invention are disclosed in US patents
10 US 3226397, US 3226398, US 3226399, US 3265698, US 3267117 and US 3226385; all corresponding to the single UK patent application GB 1087325. The UK documents lists three hundred and fifteen examples of which twenty seven are specified as having anti-microbial activity.

The compounds for the use of the present invention form a selection from the
15 general formula of GB 1087325 and are those of the general class referred to in US 5097257 but distinguished therefrom by, *inter alia*, particular groups at R^1 and R^2 of the formula I below. The formula I, and particularly preferred members of that formula such as those of formula II, offer compounds with advantages of selective hypoxic and anoxic potency.

20 Thus in a first aspect of the present invention there is provided the use of a compound of general formula I



R and R⁴ are independently selected from hydrogen, halogen and C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl, C₁₋₆ alkoxy, phenoxy, C₁₋₆ alkylthio, phenylthio, primary and secondary amino or hydroxy groups and

R³ is hydrogen, hydroxy, a C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl or C₁₋₆ alkoxy or haloalkoxy group for the manufacture of a medicament for the treatment of neoplasms, particularly tumours and more particularly cancer tumours,

characterised in that

R¹ is selected from a C₁₋₆ alkyl or haloalkyl group, -CO₂R⁵, where R⁵ is hydrogen or a C₁₋₆ alkyl or haloalkyl group, or a group -CH₂-X, where X is selected from groups of

formula -S-R⁶, -O-R⁶ and -N^{R⁷}_{R⁸} where R⁶ is hydrogen or a leaving group the acid HR⁶

of which has a pK_a of 10 or less and R⁷ and R⁸ are the same or different and are selected from C₁₋₆ alkyl or haloalkyl or together with the interjacent nitrogen form a heterocyclic

ring of 5 to 7 atoms optionally substituted by a C₁₋₄ alkyl group or haloalkyl

and

R² is selected from hydrogen, C₁₋₄ alkyl and haloalkyl and -(CH₂)_nCHR⁹R¹⁰ of more than four carbons where n is an integer of 0 to 2 and R⁹ and R¹⁰ are independently selected from a C₁₋₄ alkyl or haloalkyl group, or R⁹ and R¹⁰ together with the interjacent carbon

atom form a C₃₋₇ cycloalkyl or cycloalkenyl ring optionally substituted with one or more C₁₋₄ alkyl or haloalkyl, or C₂₋₄ alkenyl or haloalkenyl groups.

In a preferred use of the invention R is a C₁₋₆ alkoxy, haloalkoxy, C₂₋₄ alkenoxy or haloalkenoxo, phenoxy, C₁₋₆ alkylthio, phenylthio, primary or secondary amino or hydroxy group; R³ is preferably hydrogen, hydroxy, a C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl or C₁₋₆ alkoxy group and R⁴ is preferably hydrogen, halogen or a C₁₋₆ alkyl or haloalkyl, C₂₋₄ alkenyl or haloalkenyl group.

Preferred salts are suitable for direct administration to patients and thus are pharmaceutically or 'physiologically' acceptable salts. Such salts may be made with a compound described above and a physiologically acceptable acid or, where the compound contains a suitable negatively chargeable group, base. Thus salts may be formed with various inorganic and organic acids. Examples of these acids are phosphonic, nitric,

sulphuric, hydrohalic, citric, oxalic, fumaric, maleic, lactic, succinic, malic, tartaric and methane sulphonic acids. Preferred halide salts are those of hydrochloric, hydrobromic or hydroiodic acid. Examples of bases are alkali metal hydroxides and quaternary ammonium hydroxides eg. 2-amino-2-hydroxymethyl propane-1,3-diol (Tris) salts.

5 R is preferably selected from C₁₋₄ alkoxy, C₁₋₄ haloalkoxy, C₂₋₄ alkenoxy, C₂₋₄ haloalkenoxo and aziridin-1-yl groups, the aziridin-1-yl groups being optionally substituted with C₁₋₄ alkyl, or C₁₋₄ haloalkyl or C₂₋₄ alkenyl or C₂₋₄ haloalkenyl.

R² is preferably selected from C₁₋₄ n-alkyl or -haloalkyl groups or groups of formula -(CH₂)_nCHR⁹R¹⁰, where n is an integer from 0 to 2, where R⁹ and R¹⁰ are
10 independently selected from C₁₋₄ alkyl and C₁₋₄ haloalkyl or together with the interjacent carbon atom form a C₃₋₆ cycloalkyl or cycloalkenyl group optionally substituted with C₁₋₄ alkyl, C₁₋₄ haloalkyl, C₂₋₄ alkenyl or C₂₋₄ haloalkenyl.

Preferably R² is a C₁₋₄ n-, iso- or cyclo-alkyl group, particularly a methyl or cyclopropyl group⁵.

15 R³ is more preferably selected from C₁₋₄ alkyl or haloalkyl and C₂₋₄ alkenyl and haloalkenyl groups.

R⁴ is more preferably selected from hydrogen, C₁₋₄ alkyl and haloalkyl groups and most preferably is hydrogen.

Particularly preferred compounds are those where R is aziridin-1-yl optionally
20 substituted with C₁₋₄ alkyl or C₁₋₄ haloalkyl or where R is C₁₋₄ alkoxy.

One group of preferred compounds are those where the group R¹ is selected from -CH₂-O-R⁶ and -CH₂-S-R⁶ where R⁶ is hydrogen or where R⁶ is an optionally substituted phenyl or benzyl group or a group -C(O)-R¹¹ where R¹¹ is an optionally substituted phenyl, benzyl or amino group; examples of such groups R¹ including alkoxy,
25 aryloxycarbonyloxy, aryloxycarbonyloxy and carbamoyloxy. Optional substituents include halo, C₁₋₆ alkyl or haloalkyl, nitro and sulfo groups. Conveniently R¹ is hydroxymethyl.

Still more preferred compounds are those wherein R¹ is a group -CH₂X where X
30 is $\begin{array}{c} \text{R}^7 \\ \diagup \text{N} \diagdown \\ \text{R}^8 \end{array}$ wherein R⁷ and R⁸, together with the interjacent nitrogen form a 5 to 7 membered

heterocyclic ring containing nitrogen and carbon with optional oxygen or sulphur members, e.g. piperazine, morpholine, thiomorpholine rings. More preferably the heterocyclic ring is a piperazinyl ring optionally substituted by one or more C_{1-4} alkyl or haloalkyl groups. It is particularly preferred that X is a 4-alkylpiperazin-1-yl group, eg. a 4-methylpiperazine group.

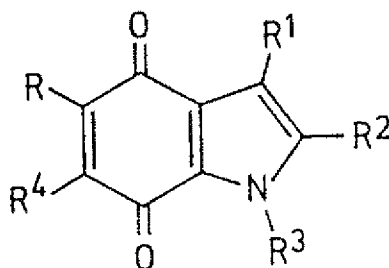
R^3 is most preferably a C_{1-4} alkyl group and R^4 is most preferably hydrogen.

Particularly preferred compounds include those where R is aziridin-1-yl optionally substituted with C_{1-4} alkyl, eg. 2-methylaziridin-1-yl, R^2 is cyclopropyl or isopropyl or C_{1-4} n-alkyl, R^3 is methyl and R^4 is hydrogen.

The most preferred compounds for the use of the invention are those which have a $C_{50}(Air)\mu M/C_{50}(N_2)\mu M$ ratio of 40 or more, still more preferably of 100 or more. No particular upper limit applies to preferred compounds as a relatively selective action against hypoxic or anoxic cells is desired. It is also preferred that the compounds have a hypoxic potency such that their $C_{50}(N_2)$ value is less than $10\mu M$, more preferably less than $1\mu M$ and most preferably less than $0.01\mu M$. Typically this is in the range 0.005 to $1\mu M$.

Most preferred compounds are described herein as compounds 21, 54 and 84 of the Examples described below; the numbers being in brackets at the end of the example title.

In a second aspect of the invention there are provided novel compounds falling within formula I above. Particular novel compounds of the invention are those of formula Ia below. Thus this second aspect of the present invention provides a novel compound of formula Ia



or a salt thereof

characterised in that

- R is C₁₋₄ alkoxy or haloalkoxy or aziridin-1-yl optionally substituted with one or more C₁₋₄ alkyl or haloalkyl groups,
 R⁴ is hydrogen or a C₁₋₄ alkyl or haloalkyl group,
 R³ is hydrogen, hydroxy, C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl or C₁₋₆ alkoxy or haloalkoxy,
 R¹ is -CO₂R⁵ where R⁵ is a hydrogen or C₁₋₆ alkyl or haloalkyl group, or is a group -CH₂X where X is selected from -S-R⁶, -O-R⁶ and -NR⁷R⁸ where R⁶ is hydrogen or a leaving group the acid HR⁶ of which has a pKa of 10 or less and R⁷ and R⁸ are the same or different and are selected from C₁₋₆ alkyl or haloalkyl or together with the interjacent nitrogen form a heterocyclic ring of 5 to 7 atoms optionally substituted by a C₁₋₄ alkyl or haloalkyl group and
 R² is hydrogen or a C₁₋₄ alkyl group or a group -(CH₂)_nCHR⁹R¹⁰ of more than four carbons where n is an integer of 0 to 2 and R⁹ and R¹⁰ are independently selected from C₁₋₄ alkyl and haloalkyl or R⁹ and R¹⁰ together with the interjacent carbon atom form a C₃₋₇ cycloalkyl or cycloalkenyl ring optionally substituted with one or more C₁₋₄ alkyl or haloalkyl or C₂₋₄ alkyl or haloalkyl groups;
 with the provisos that
 (i) when R is methoxy, R¹ is hydroxymethyl or a carbamate or C₁₋₆ aliphatic ester thereof, R⁴ is methyl or ethyl and R³ is methyl, optionally 2-substituted ethyl, propyl or butyl, then R² is not hydrogen, methyl, fluoromethyl, chloromethyl or ethyl,
 (ii) when R is methoxy, R⁴ is hydrogen, R¹ is hydroxymethyl or the propylcarbamate thereof and R³ is ethyl, then R² is not methyl,
 (iii) when R is methoxy, R² is methyl, R³ is ethyl and R⁴ is methyl, then R¹ is not hydroxymethyl cyclohexylcarboxylate, benzoate, furanyl-2-carboxylate, 3-(2-dimethyl-aminoethyl)-piperazine-1-carboxylate, morphalinocarbamate, 4-(3-hydroxypropyl)-piperazinecarbamate or 4-(3-dimethylaminopropyl)-piperazinecarbamate,
 and
 (iv) when R is aziridinyl or ethoxy, R¹ is hydroxymethyl or a carbamate or C₁₋₆ aliphatic ester thereof, R⁴ is methyl or bromo and R³ is ethyl, then R² is not methyl.

Preferred novel compounds of the invention are those where R^1 is a hydroxymethyl group or a group $-CH_2-X$ as defined above. Most preferred novel compounds are those where R is aziridin-1-yl or 2-alkyl-aziridin-1-yl, such as 2-methyl-aziridin-1-yl.

R^3 is preferably C_{1-4} alkyl or haloalkyl and R^2 is preferably n- C_{1-4} alkyl, isopropyl or C_{3-7} cycloalkyl. R^4 is preferably methyl or hydrogen, most preferably hydrogen.

A third aspect of the present invention provides a novel compound of the second aspect of the invention or a physiologically acceptable salt thereof for use as a therapeutic agent.

10 A preferred use as therapeutic agent as provided by this invention is for the treatment of tumour cells, and most preferably the use is that which administers the compound in treatment also comprising radiation treatment, e.g. with therapeutic radiation such as X-rays, γ -rays and electrons from an accelerator, e.g. linear accelerator. The use of the compound with radiation is particularly effective for achieving cytostasis and/or death of
15 tumour cells, particularly of cancer cells. Most preferably the use is aimed at providing cytotoxicity of anoxic tumour cells, ie. those relatively poorly supplied with blood.

Particularly the use is aimed at providing cytotoxicity towards hypoxic and anoxic tumour cells which, at the time of treatment, exist at an oxygen tension of less than 7.6 mm Hg of O_2 (1%).

20 A fourth aspect of the present invention provides compositions comprising a novel compound of the second aspect of the invention or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier. Compositions will preferably be provided as discrete dosage units and packs thereof, e.g. in a form suitable for parenteral or oral administration. Other suitable dosage unit forms will occur to those skilled in the art.

25 A fifth aspect of the present invention provides a method of treating a human or animal body for the purposes of killing, and/or preventing growth of, tumour cells comprising administering a medicament manufactured with the compound of the first aspect of the invention, or a pharmaceutically acceptable salt thereof, to the body in a dose sufficient to kill or prevent growth of some or all of the tumour cells.

Preferred methods of the fifth aspect are for treating hypoxic and anoxic tumour cells, particularly cancer cells. It will be realised that the treatment will usually include killing of oxic cells, but that in preferred forms the ratio of such cells killed as compared with anoxic or hypoxic cells will be relatively low.

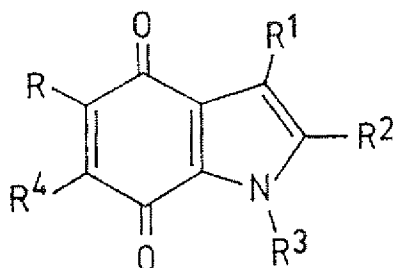
5 A still more preferred method of the fifth aspect comprises administering the medicament manufactured with the compound or salt of the first aspect to the body within a set period of treatment of the body with doses of radiation, the doses of radiation being sufficient to kill or prevent growth of some or all of any oxic tumour cells eg. those relatively well supplied with blood. This combination treatment allows targeted radiation
10 to be used to damage and/or kill the oxic tumour cells while the systemically administered compound of the invention is used to damage or kill the hypoxic and anoxic cells selectively, thus causing reduced peripheral damage to healthy non-tumour cells.

The compounds of the present invention are of the indoloquinone class and as such will have application in treatment of tumours such as those vulnerable to mitomycins and
15 the compounds disclosed in US 5079257. These include all solid tumour types, for example cervical, breast, pancreatic and prostatic adenocarcinoma, colon, bladder, gastro-intestinal cancers and melanomas. Such tumours may exist in an area of hypoxia and contain the reductive enzymes necessary to activate the agents for the use of this invention.

20 A sixth aspect of the invention provides novel compounds that have utility as intermediates for use in the preparation of a compound of the first aspect of the invention, these intermediates being novel compounds as described in the Figures 2 to 5 and the Examples 1 to 49.

Particularly preferred novel intermediates are those of formula II below

25



Formula II

wherein R is C₁₋₄ alkoxy, R¹ is C₁₋₄ alkoxycarbonyl and R², R³ and R⁴ are as for formula Ia. It will be realised that some of these compounds will also be compounds of the first aspect of the invention but, where these have an HCR of less than 40, and particularly have an HCR of less than 10, their main interest will be as intermediates for use in synthesis of the more preferred compounds. In this respect the 5-alkoxy compounds will usually have a less favourable efficacy and HCR ratio than the corresponding aziridinyl compounds and thus their prime use will be as intermediates. Preferred intermediates are those where R is methoxy, R¹ is methoxycarbonyl and R³ is methyl.

A seventh aspect of the present invention provides a process for preparing compounds of formula Ia and II.

In a first preferred process of this aspect there is provided a process for preparing an optionally substituted aziridin-1-yl compound of formula II where R¹ is alkoxycarbonyl comprising reacting a corresponding 2-substituted-3-alkoxycarbonyl-5-alkoxy-1-alkyl-indole-4,7-dione with an optionally substituted aziridine.

In a second preferred process for preparing a compound of formula Ia where R¹ is a hydroxymethyl group a corresponding 2-substituted-3-alkoxycarbonyl-5-alkoxy-1-alkyl-indole-4,7-dione is reacted with an oxidising reagent, such as Na₂S₂O₄, then the resultant hydroquinone is reduced, e.g. with DIBAL-H, to the product.

For all aspects of the present invention halo is preferably fluoro or chloro.

The compounds, compositions, use, methods and intermediates of the invention will now be described by way of illustration only by reference to the following non-limiting Figures and Examples. Further embodiments of the invention will occur to those skilled in the art in the light of these.

Figures.

Figure 1: Shows the chemical structure of some known compounds referred to in the specific description above as compounds 1 to 5.

Figure 2: Shows the outline of the synthetic route used to prepare compounds 10, 11, 12 and 27, 28, 29 referred to herein.

Figure 3: Shows the outline of the synthetic route used to prepare compounds 13 to 42 referred to herein.

Figure 4: Shows the outline of the synthetic route used to prepare compounds 47 to 57 referred to herein.

5 Figure 5: Shows the outline of the synthetic route used to prepare compounds 58 to 66 referred to herein.

Figure 6: Is a graph plotting mean relative surviving RIF-1 tumour cell fraction against a varying dose of compound 21 alone and against varying dose of compound 21 administered after 15 GY of radiation. The surviving fraction after 15 GY radiation alone
10 is shown by a flat line.

Figure 7: Is a graph plotting mean relative surviving RIF-1 tumour cell fraction under conditions as set out for Figure 6 but using compound 54 as test compound.

Figure 8: Is a graph plotting mean relative surviving KHT tumour cell fraction against a
15 varying dose of compound 21 alone and against a varying dose of compound 21 administered after 10 GY of radiation. The surviving fraction after 10 GY radiation alone is shown by a flat line.

Figure 9: Is a graph plotting mean relative surviving KHT tumour cell fraction under
20 conditions as set out for Figure 8 but using compound 54 as test compound.

Definitions of the groups used in the Figures can be found in the text of Examples below and in the Figures themselves.

EXAMPLES

General methods for preparation of compounds 18 to 84:

2-Alkyl and cycloalkyl substituted indoles were synthesized in 14 steps from the common precursor 3-chlorophenol as shown in Figure 2 and 3. The crucial step was the
5 1,5-electrocyclisation of the imine (e.g. compound 8) to the 2,3-dihydroindole derivatives, which was successfully carried out using isobutyraldehyde, cyclohexane carboxaldehyde or cyclopropane carboxaldehyde, but not with acrolein in a proposed alternative route, using zinc acetate in methanol as has been employed in the synthesis of EO9 via a 2-acrylate derivative in WO 87/06227. Nitration at the desired 4-position
10 could be achieved only subsequent to the N-methylation step in order to avoid increasing yields of the 6-nitro isomer. The subsequent 6 steps, including nitration, oxidations (DDQ and Fremy's salt) and reductions (Sn/HCl, LiAlH₄, Na₂S₂O₄ and DIBAL-H) left the 2-cyclopropyl moiety intact and the desired indoloquinones were obtained. Substitution of the 5-methoxy substituent was successful in high yielding
15 reactions with aziridine and 2-methylaziridine.

Comparable 1,2-dimethyl analogues 52-56 were synthesised from commercially available 2-methyl-5-methoxyindole in 7 steps (Figure 4). Substitution of the 5-position with aziridine and 2-methylaziridine was again successful in high yield, as was the *cis*-2,3-dimethylaziridine. However 2,2-dimethylaziridine reacted with
20 difficulty and the resulting 5-(2,2-dimethylaziridinyl) analogue was unstable in aqueous solution and on silica gel, ring-opening via an S_N1 mechanism to give 57. The 2-unsubstituted analogues 62-66 were obtained in 6 steps from 5-methoxyindole-3-carboxaldehyde (Figure 5).

NMR spectra were obtained at 90 MHz with a JEOL FX90Q spectrometer
25 using SiMe₄ as internal standard. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, Middlesex, U.K. Solutions in organic solvents were dried by treatment with MgSO₄ or Na₂SO₄ and filtration. Dichloromethane (CH₂Cl₂) was dried over calcium chloride and passed through neutral alumina prior to use. Dimethylformamide (DMF), toluene and tetrahydrofuran (THF)
30 were anhydrous commercial grades. Silica gel for flash column chromatography was

Merck grade (230-400 mesh). Melting points were determined on a Thomas Hoover melting point apparatus and on a Thermogallen Microscope and Hot Stage Apparatus and are uncorrected. Stereochemically pure *cis*-2,3-dimethylaziridine and 2,2-dimethylaziridine were synthesized from the appropriately substituted 2-aminoethanols by

- 5 *O*-sulfation and elimination with KOH according to the methods of Dickey *et al.* (see reference 19). Fused cyclopropamitosenes 4 and 5 were synthesized as described by Cotterill *et al.* (see reference 1) and O'Sullivan (see reference 20). Starting materials 2-chlorophenol, 5-methoxy-2-methylindole and 5-methoxyindole-3-carboxaldehyde were all purchased from Sigma-Aldrich Chemical Company.

- 10 **Example 1: Preparation of 2-Cyclopropyl-3-methoxycarbonyl-5-methoxy-1-methylindole-4,7-dione (18) by reactions (v) to (xvii) of Figure 2.**

(a) Diethyl 5-Methoxy-2-nitrophenylmalonate (6)

- A solution of 17 g (0.064 mol) of ethyl 5-methoxy-2-nitrophenylcyanoacetate, prepared in 4 steps from 3-chlorophenyl as described by Speckamp and Oostveen
15 (WO 87/06227), in EtOH (100 mL), was saturated with HCl gas on cooling in an ice/salt bath. The solution was stirred for 2 days and then ice-water (100 mL) added and the solution stirred for a further 24 hours at 4°C before the crystalline solid formed was collected by filtration, dried and recrystallized from EtOH to give 17 g (85%) of 6 as a white solid: mp 92-93°C (Lit. (Reference 4) 92-93°C).

- 20 **(b) 2-Cyclopropyl-3,3-diethoxycarbonyl-2,3-dihydro-5-methoxyindole (9).**

- Compound 6 (5.0 g, 16.1 mmol) was dissolved in a mixture of toluene (62.5 mL) and EtOH (3.75 mL) and reduced with H₂ at atmospheric pressure over PtO₂ (75 mg) catalyst. After 7 hours at room temperature the solution was filtered through Celite and evaporated to dryness BELOW 30°C, and the resulting pale green oil (7) dissolved
25 immediately in MeOH (75 mL). To this methanolic solution was added cyclopropane carboxaldehyde (1.3 g, 18.6 mmol) dissolved in MeOH (10 mL) and the solution stirred for 15 minutes. The resulting solution of imine 8 was treated *in situ* with Zn(OAc)₂·2H₂O (1.13 g, 5.15 mmol) and stirred for 18 hours at room temperature.

The solution was evaporated to dryness at 30°C, HCl (2.0 M, 50 mL) added and extracted with CH₂Cl₂ (2 x 150 mL), washed with sat. NaHCO₃ (aq., 150 mL), sat. NaCl (aq., 100 mL), dried and evaporated. The residue was purified on silica, eluting with hexane/EtOAc (1:1, R_f=0.75) to give 9 (3.3 g, 68%) as a yellow oil:

5 ¹H-NMR (CDCl₃) δ 0.48–0.52 (m, 4H, 2 x cyclopropyl-CH₂), 1.03–1.08 (m, 1H, cyclopropyl-H), 1.2 (t, 6H, J=7.2 Hz, CH₂CH₃), 1.3 (t, 6H, J=7.2 Hz, CH₂CH₃), 3.75 (s, 3H, CH₃O-), 3.85 (d, 1H, J=2.7 Hz, 2-H), 4.1–4.35 (m, 4H, CH₂CH₃), 6.63 (s, 1H, Ar-4H), 6.71 (d, 1H, J=1.5 Hz, Ar-6H) and 7.03 (d, 1H, J=1.5 Hz, Ar-7H).

(c) 1-Acetyl-2-cyclopropyl-3,3-diethoxycarbonyl-2,3-dihydro-5-methoxyindole

10 (10).

Compound 9 (3.0 g, 10 mmol) was dissolved in Ac₂O (5 mL) and stirred for 3 hours at room temperature. The anhydride was then evaporated *in vacuo* and the residue purified on silica, eluting with hexane/EtOAc (2:1, R_f=0.4) to give a pale yellow oil which was redissolved in Et₂O and evaporated. Trituration of the resulting oil with Et₂O gave a

15 white solid which was collected by filtration and washed with cold Et₂O to give 10 (3.0 g, 87%) as a white solid: mp 104–105°C; ¹H-NMR (CDCl₃) δ 0.5–0.57 (m, 4H, 2 x cyclopropyl-CH₂), 1.0–1.15 (m, 1H, cyclopropyl-H), 1.2 (t, 3H, J=7.2 Hz, CH₂CH₃), 1.3 (t, 3H, J=7.2 Hz, CH₂CH₃), 3.81 (s, 3H, CH₃O-), 4.05–4.3 (m, 4H, CH₂CH₃), 4.5 (d, 1H, J=2.7 Hz, 2-H) and 6.9–7.2 (m, 3H, Ar-4,6,7H).

20 (d) 1-Acetyl-3-carboxy-2-cyclopropyl-2,3-dihydro-5-methoxyindole (11).

The acetylindole 10 (1.0 g, 2.9 mmol) was dissolved in EtOH (10 mL) and cooled to 0°C in an ice-salt bath, and a cold (0°C) solution of KOH (aq., 10%, 10 mL) was added. The solution was stirred at -5°C for 4 hours and then for 18 hours at 4°C. The solution was poured onto ice/water (25 mL) and washed with Et₂O. The aqueous layer was

25 then acidified with 2.0 M HCl and extracted with CHCl₃ (6 x 50 mL), dried and evaporated to give 0.68 g (97%) of 11 as a yellow oil (R_f=0.45, Me₂CO), which was used in the next step without further purification; ¹H-NMR (CDCl₃) δ 0.5–0.7 (m, 4H, 2 x cyclopropyl-CH₂), 1.25–1.4 (m, 1H, cyclopropyl-H), 2.33 (s, 3H, COCH₃), 3.78 (s, 3H, CH₃O-), 3.85 (br, 1H, 3-H), 4.5 (br s, 1H, 2-H), 6.9–7.0 (m, 2H, Ar-4,6H),
30 8.05–8.15 (m, 1H, Ar-7H) and 10.81 (s, 1H, CO₂H).

(e) Methyl 1-acetyl-2-cyclopropyl-2,3-dihydro-5-methoxyindole-3-carboxylate (12).

To a solution of 11 (0.68 g, 2.8 mmol) in DMF (10 mL) was added K_2CO_3 (0.83 g, 6 mmol) and $(MeO)_2SO_2$ (2 g, 15.8 mmol) and the solution stirred at room temperature for 4 hours. The solution was then poured onto HCl (2.0 M, 20 mL) and extracted with $CHCl_3$ (4 x 25 mL), washed with sat. NaCl, dried and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:2, $R_f=0.7$ (EtOAc)) to give a pale yellow oil of 12 (0.7g, 98%), which solidified on standing; 1H -NMR ($CDCl_3$) δ 0.58–0.64 (m, 4H, 2 x cyclopropyl- CH_2), 1.25–1.33 (m, 1H, cyclopropyl- H), 2.33 (s, 3H, $COCH_3$), 3.68 (s, 3H, CO_2CH_3), 3.79 (s, 3H, CH_3O -), 3.85 (br, 1H, 3- H), 4.54 (br, 1H, 2- H), 6.78–6.95 (m, 2H, Ar-4, 6H) and 8.2 (br s, 1H, Ar-7H).

(f) Methyl 1-acetyl-2-cyclopropyl-5-methoxyindole-3-carboxylate (13).

A solution of 12 (1.0 g, 4 mmol) was stirred under reflux with DDQ (0.96 g, 4.2 mmol) in toluene (12.5 mL) for 7 hours. The DDQH₂ was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified on silica, eluting with hexane/EtOAc (1:1, $R_f=0.7$) to give 13 (0.88 g, 87%) as a pale yellow oil; 1H -NMR ($CDCl_3$) δ 0.73–0.79 (m, 2H, cyclopropyl- CH_2), 1.22–1.3 (m, 2H, cyclopropyl- CH_2), 2.15–2.25 (m, 1H, cyclopropyl- H), 2.83 (s, 3H, $COCH_3$), 3.86 (s, 3H, CO_2CH_3), 3.97 (s, 3H, CH_3O -), 6.99 (dd, 1H, $J=2.7$ and 9 Hz, Ar-6H), 7.5 (d, 1H, $J=2.7$ Hz, Ar-4H) and 7.96 (d, 1H, $J=9$ Hz, Ar-7H).

(g) Methyl 2-cyclopropyl-5-methoxyindole-3-carboxylate (14).

A solution of 13 (0.88 g, 3.46 mmol) in KOH (4% in MeOH, 50 mL) was stirred at room temperature for 1.5 hours, neutralized with 6.0M HCl and extracted with EtOAc (3 x 25 mL). The organic layer was washed with H₂O (50 mL), dried and evaporated to give 14 (0.51 g, 70%) as a white solid: mp 128–131 °C; 1H -NMR ($CDCl_3$) δ 0.82–0.92 (m, 2H, cyclopropyl- CH_2), 1.05–1.2 (m, 2H, cyclopropyl- CH_2), 2.2–3.0 (m, 1H, cyclopropyl- H), 3.85 (s, 3H, CO_2CH_3), 3.95 (s, 3H, CH_3O -), 6.79 (dd, 1H, $J=2.7$

and 9 Hz, Ar-6H), 7.22 (d, 1H, J=6.3 Hz, Ar-7H), 7.58 (d, 1H, J=2.7 Hz, Ar-4H) and 8.2 (br s, 1H, NH).

(h) Methyl 2-cyclopropyl-5-methoxy-1-methylindole-3-carboxylate (15).

Compound 14 (9.0 g, 42.6 mmol) was added under argon to a stirred suspension of
5 NaH (6.0 g, 0.13 mol) in DMF (150 mL). The solution was heated at 45°C for
0.5 hours, cooled to 0-10°C and MeI (33 mL, 0.23 mol) added. The solution was then
gradually heated to 60°C and stirred at this temperature for 1 hour, cooled and poured
onto cold (0°C) NaHSO₄ (aq., 10%, 500 mL) and extracted with EtOAc (5 x 75 mL).
The organic extracts were washed with sat. NaCl (150 mL), dried and evaporated. The
10 residue was purified on silica, eluting with EtOAc/hexane (1:2, R_f=0.45) to give 15
(8.3 g, 86%) as a pale yellow waxy solid; ¹H-NMR (CDCl₃) δ 0.77-0.84 (m, 2H,
cyclopropyl-CH₂), 1.21-1.29 (m, 2H, cyclopropyl-CH₂), 1.85-2.05 (m, 1H,
cyclopropyl-H), 3.8 (s, 3H, CH₃N-), 3.86 (s, 3H, CO₂CH₃), 3.9 (s, 3H, CH₃O-),
6.86 (dd, 1H, J=2.7 and 9 Hz, Ar-6H), 7.23 (d, 1H, J=9 Hz, Ar-7H) and 7.62 (d, 1H,
15 J=2.7 Hz, Ar-4H).

(i) Methyl 1-methyl-2-cyclopropyl-5-methoxy-4-nitroindole-3-carboxylate (16).

To a solution of 15 (8.0 g, 34.66 mmol) in AcOH (150 mL) cooled to 0°C, was added
dropwise a cold (0°C) mixture of f.HNO₃ (27 mL) in AcOH (100 mL). The solution
was stirred for 3 hours while allowing to reach room temperature, and then poured onto
20 300 g of crushed ice and after 15 minutes stirring the resulting yellow solid collected by
suction filtration. The dried residue was purified on silica, eluting with EtOAc/hexane
(1:1, R_f=0.45) to give 7.5 g (71%) of 16 as a yellow solid, recrystallised from EtOAc:
mp 129-131°C; ¹H-NMR (CDCl₃) δ 0.74-0.81 (m, 2H, cyclopropyl-CH₂), 1.22-1.3
(m, 2H, cyclopropyl-CH₂), 1.85-2.05 (m, 1H, cyclopropyl-H), 3.8 (s, 3H, CH₃N-),
25 3.81 (s, 3H, CO₂CH₃), 3.89 (s, 3H, CH₃O-), 6.94 (d, 1H, J=9 Hz, Ar-7H) and
7.32 (d, 1H, J=9 Hz, Ar-6H).

(j) Methyl 4-amino-2-cyclopropyl-5-methoxy-1-methylindole-3-carboxylate (17).

To a suspension of 2.5 g (9.25 mmol) 16 in EtOH (180 mL) were added tin powder (5.25 g, 44.2 mmol) and HCl (3.0 M, 70 mL) and the solution stirred at room temperature for 1 hour. The solution was then decanted from the excess tin and

- 5 neutralized with sat. NaHCO_3 (aq.) and the resulting red suspension added to an equal volume of H_2O and extracted with CHCl_3 (5 x 50 mL) and then EtOAc (5 x 50 mL) and the combined extracts evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, $R_f=0.5$) to give 17 (2.2 g, 87%) as a white solid which was used immediately in the next step: mp 49-50°C; $^1\text{H-NMR}$ (CDCl_3) δ 0.65-0.72 (m, 2H, cyclopropyl- CH_2), 1.22-1.3 (m, 2H, cyclopropyl- CH_2), 1.8-1.9 (m, 1H, cyclopropyl- H), 3.73 (s, 3H, $\text{CH}_3\text{N-}$), 3.85 (s, 3H, CO_2CH_3), 3.9 (s, 3H, $\text{CH}_3\text{O-}$), 5.6 (br s, 1H, NH_2), 6.5 (d, 1H, $J=9$ Hz, Ar-7H) and 6.9 (d, 1H, $J=9$ Hz, Ar-6H).

(k) 2-Cyclopropyl-3-methoxycarbonyl-5-methoxy-1-methylindole-4,7-dione (18).

To a solution of 17 (2.0 g, 7.2 mmol) in Me_2CO (250 mL) was added a solution of potassium nitrosodisulfonate ($(\text{KSO}_3)_2\text{NO}$, Fremy's salt, 9.7 g, 36.2 mmol) in $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer (250 mL, 0.3 M, pH 6.0) and the solution stirred at room temperature for 1 hour. The Me_2CO was removed *in vacuo* and the resulting orange precipitate collected by suction filtration, washed with H_2O and dried in a vacuum oven at 45°C to afford 18 as an orange solid, recrystallized from EtOAc: mp 169-170°C;

- 20 $^1\text{H-NMR}$ (CDCl_3) δ 0.69-0.77 (m, 2H, cyclopropyl- CH_2), 1.1-1.2 (m, 2H, cyclopropyl- CH_2), 1.48-1.58 (m, 1H, cyclopropyl- H), 3.80 (s, 3H, $\text{CH}_3\text{N-}$), 3.90 (s, 3H, CO_2CH_3), 4.01 (s, 3H, $\text{CH}_3\text{O-}$) and 5.64 (s, 1H, 6-H). Anal. C; 62.06, H; 5.20, N; 4.71%, Calc. ($\text{C}_{15}\text{H}_{15}\text{NO}_5$) C; 62.28, H; 5.19, N; 4.84%.

Example 2: 5-(Aziridin-1-yl)-2-cyclopropyl-3-methoxycarbonyl-1-methylindole-4,7-dione (19) by reaction (xviii) of Figure 2.

25 Compound 18 (0.29 g, 1 mmol) was dissolved and stirred in freshly redistilled 1(H)-aziridine (3 mL, ca. 70 mmol, CAUTION!) for 1 hour, evaporated *in vacuo* and the residue redissolved in EtOAc. The solvent was then partially evaporated until a red precipitate appeared. The red solid was then collected by suction filtration and

recrystallized from EtOAc to afford 19 (0.25 g, 83%) as a red solid: mp 138-140°C; ¹H-NMR (CDCl₃) δ 0.65-0.72 (m, 2H, cyclopropyl-CH₂), 1.2-1.35 (m, 2H, cyclopropyl-CH₂), 1.7-1.8 (m, 1H, cyclopropyl-H), 2.18 (s, 4H, 2 x azir-CH₂), 3.92 (s, 3H, CH₃N-), 3.99 (s, 3H, CO₂CH₃) and 5.77 (s, 1H, 6-H). Anal. C; 63.60, H; 5.41, N; 9.33%, Calc. (C₁₆H₁₆N₂O₄) C; 64.00, H; 5.33, N; 9.33%.

Example 3: 2-Cyclopropyl-3-hydroxymethyl-5-methoxy-1-methylindole-4,7-dione (20) by reaction (xix) of Figure 2.

To a solution of 18 (0.3 g, 1.03 mmol) in CHCl₃ (30 mL) and EtOH (11 mL) was added a solution of Na₂S₂O₄ (2.1 g, 12 mmol) in H₂O (13 mL). The solution was stirred at room temperature for 0.5 hour and the organic layer separated, washed with sat. NaCl (50 mL), dried and evaporated. The crude hydroquinone was then dissolved in anhydrous CH₂Cl₂ (30 mL) under argon and cooled to -30°C, and DIBAL-H (5 mL of a 1.5 M solution in toluene) added dropwise such that the solution temperature remained BELOW -30°C. The solution was then allowed to reach 0°C and stirred for 2.5 hours at this temperature, and a solution of FeCl₃ (9 mL, 1.0 M (0.1 M HCl)) added. The solution was stirred for 10 minutes at 0°C and then CHCl₃ (150 mL) and H₂O (150 mL) added. The aqueous layer was extracted with CHCl₃ (5 x 50 mL) then EtOAc (5 x 50 mL) and the combined organic phases washed with sat. NaCl (250 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc (R_f=0.5) to give 20 as an orange solid after recrystallization from EtOAc (125 mg, 47%): mp 200-202°C; ¹H-NMR (CDCl₃) δ 0.71-0.82 (m, 2H, cyclopropyl-CH₂), 1.2-1.33 (m, 2H, cyclopropyl-CH₂), 1.61-1.71 (m, 1H, cyclopropyl-H), 3.81 (s, 3H, CH₃N-), 3.98 (s, 3H, CH₃O-), 4.0 (br s, 1H, CH₂OH), 4.69 (br d, 2H, CH₂OH) and 5.64 (s, 1H, 6-H). Anal. C; 64.00, H; 5.66, N; 5.12%, Calc. (C₁₄H₁₅O₄) C; 64.36, H; 5.75, N; 5.36%.

Example 4: 5-(Aziridin-1-yl)-2-cyclopropyl-3-hydroxymethyl-1-methylindole-4,7-dione (21) by reaction (xx) of Figure 2.

Compound 20 (0.1 g, 0.38 mmol) was dissolved and stirred in freshly distilled (CAUTION!) 1(H)-aziridine (3 mL, ca. 70 mmol) for 0.75 hour, evaporated *in vacuo* and the residue redissolved in EtOAc, evaporated until a red precipitate appeared and the solid collected. The red solid was recrystallized from EtOAc to give 21 (80 mg, 77%): mp 177-179.5°C; ¹H-NMR (CDCl₃) δ 0.7-0.8 (m, 2H, cyclopropyl-CH₂), 1.22-1.3 (m, 2H, cyclopropyl-CH₂), 1.6-1.7 (m, 1H, cyclopropyl-H), 2.18 (s, 4H, 2 x azir-CH₂), 3.97 (s, 3H, CH₃N-), 4.73 (s, 2H, CH₂OH) and 5.77 (s, 1H, 6-H). Anal. C; 66.35, H; 5.61, N; 10.26%, Calc. (C₁₅H₁₆N₂O₃) C; 66.18, H; 5.88, N; 10.29%.

Example 5: 2-Cyclopropyl-3-hydroxymethyl-5-(2-methylaziridin-1-yl)-1-methylindole-4,7-dione (22) by reaction (xx) of Figure 2.

Compound 20 (0.1 g, 0.38 mmol) was dissolved and stirred in freshly distilled 2-methylaziridine (3 mL, ca. 50 mmol) for 2.5 hours. The solution was evaporated *in vacuo* and the residue redissolved in EtOAc, evaporated until a red precipitate appeared and the solid collected. The red solid was recrystallized from EtOAc to give 22 (85 mg, 78%): mp 130-131°C; ¹H-NMR (CDCl₃) δ 0.68-0.74 (m, 2H, cyclopropyl-CH₂), 1.08-1.2 (m, 2H, cyclopropyl-CH₂), 1.42 (d, 3H, J=4.5 Hz, azir-CH₃), 1.5-1.6 (m, 1H, cyclopropyl-H), 2.01-2.15 (m, 3H, azir-CHCH₂), 3.97 (s, 3H, CH₃N-), 4.73 (s, 2H, CH₂OH) and 5.74 (s, 1H, 6-H). Anal. C; 67.12, H; 6.04, N; 9.57%, Calc. (C₁₆H₁₈N₂O₃) C; 67.13, H; 6.29, N; 9.79%.

Example 6: 2-Cyclopropyl-5-methoxy-1-methyl-3-[[[(phenoxycarbonyl)oxy]methyl]indole-4,7-dione (23) by reaction (xxi) of Figure 2.

To a solution of 20 (0.1 g, 0.38 mmol) in anhydrous pyridine (6 mL) at 0°C, was added dropwise phenylchloroformate (0.1 g, 0.64 mmol) and the solution then allowed to reach room temperature and stirred for 2 hours. The solution was then extracted with CH₂Cl₂ (25 mL) and washed with H₂O (25 mL) and sat. NaCl (25 mL), dried and

evaporated. The residue was purified on silica, eluting with EtOAc ($R_f=0.75$) to give 23 as an orange solid: mp 136-139°C; $^1\text{H-NMR}$ (CDCl_3) δ 1.02-1.18 (m, 2H, cyclopropyl- CH_2), 1.21-1.28 (m, 2H, cyclopropyl- CH_2), 1.78-1.88 (m, 1H, cyclopropyl- H), 3.79 (s, 3H, $\text{CH}_3\text{N-}$), 4.01 (s, 3H, $\text{CH}_3\text{O-}$), 5.27 (s, 2H, CH_2OCOPh), 5.51 (s, 1H, 6- H) and 7.15-7.3 (m, 5H, Ar).

Example 7: 2-Cyclopropyl-5-methoxy-1-methyl-3-[(carbamoyloxy)methyl] indole-4,7-dione (24) by reaction (xxii) of Figure 2.

The phenylcarbonate 23 (0.3 g, 0.78 mmol) was dissolved in anhydrous CH_2Cl_2 (38 mL) and the solution cooled to -78°C. The solution was then saturated with NH_3 and stirred at 78°C until reaction was complete (ca. 2 hours). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (100 mL) and washed with H_2O (2 x 100 mL) and sat. NaCl (50 mL), dried and evaporated, and the residue recrystallized from EtOAc to afford 220 mg (92%) of 24 as an orange solid: mp 240-242°C (dec.); $^1\text{H-NMR}$ (CDCl_3) δ 1.02-1.13 (m, 2H, cyclopropyl- CH_2), 1.22-1.26 (m, 2H, cyclopropyl- CH_2), 1.51-1.55 (m, 1H, cyclopropyl- H), 3.78 (s, 3H, $\text{CH}_3\text{N-}$), 3.99 (s, 3H, $\text{CH}_3\text{O-}$), 4.79 (br s, 2H, NH_2), 5.3 (s, 2H, $\text{CH}_2\text{OCONH}_2$) and 5.62 (s, 1H, 6- H). Anal. C; 59.42, H; 4.88, N; 8.86%, Calc. ($\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_5$) C; 59.21, H; 4.88, N; 9.21%.

Example 8: 5-(Aziridin-1-yl)-2-cyclopropyl-1-methyl-3-[(carbamoyloxy)methyl] - indole-4,7-dione (25) by reaction (xxiii) of Figure 2.

The carbamate 24 (0.3 g, 1.0 mmol) was stirred at room temperature in 1(H)-aziridine (2 mL, CAUTION!) for 15 minutes, evaporated and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume and the resulting red precipitate filtered and washed well with cold EtOAc to afford 25 (210 mg, 63%) as a red solid: mp 235-238°C(dec.); $^1\text{H-NMR}$ ($(\text{CD}_3)_2\text{SO}$) δ 0.65-0.73 (m, 2H, cyclopropyl- CH_2), 1.05-1.15 (m, 2H, cyclopropyl- CH_2), 1.73-1.85 (m, 1H, cyclopropyl- H), 2.18 (s, 4H, 2 x azir- CH_2), 3.93 (s, 3H, $\text{CH}_3\text{N-}$), 5.06 (s, 2H, $\text{CH}_2\text{OCONH}_2$), 5.78 (s, 1H, 6- H) and 6.42 (br s, 2H, NH_2). Anal. C; 60.93, H; 5.60, N; 13.38%, Calc. ($\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4$) C; 60.95, H; 5.39, N; 13.33%.

Example 9: 2-Cyclopropyl-1-methyl-5-(2-methylaziridin-1-yl)-3-[(carbamoyloxy)methyl]-indole-4,7-dione (26) by reaction (xxii) of Figure 2.

The carbamate 24 (0.05 g, 0.164 mmol) was stirred at room temperature in 2-methylaziridine (1.5 mL) for 4 hours, evaporated and redissolved in EtOAc (5 mL).

- 5 The solution was then evaporated to 50% volume and the resulting red precipitate filtered and washed well with cold EtOAc, and then recrystallized from EtOAc to afford 26 (30 mg, 56%) as a red solid: mp 209-211°C(dec.); ¹H-NMR ((CD₃)₂SO) δ 0.63-0.72 (m, 2H, cyclopropyl-CH₂), 1.01-1.09 (m, 2H, cyclopropyl-CH₂), 1.29 (d, 3H, J=5.4Hz, azir-CH₃), 1.75-1.85 (m, 1H, cyclopropyl-H), 1.98-2.05 (m, 3H, azir-CHCH₂), 3.93 (s, 3H, CH₃N-), 5.06 (s, 2H, CH₂CONH₂), 5.76 (s, 1H, 6-H) and 6.41 (br s, 2H, NH₂). Anal. C; 61.76, H; 5.41, N; 12.24%, Calc. (C₁₇H₁₉N₃O₄) C; 62.00, H; 5.77, N; 12.76%.
- 10

Example 10: Preparation of 2-Isopropyl-3-methoxycarbonyl-5-methoxy-1-methylindole-4,7-dione (35) by reactions (ix)-(xvii) of Figure 2.

- 15 (a) 1-Acetyl-3,3-diethoxycarbonyl-2,3-dihydro-2-isopropyl-5-methoxyindole (27). This compound was prepared (56%) by the method described for compound 9 but using isobutyraldehyde in place of cyclopropane carboxaldehyde. The residue after work-up was dissolved in Ac₂O (5 mL) and stirred for 3 hours at room temperature. The anhydride was then evaporated *in vacuo* and the residue purified on silica, eluting with
- 20 hexane/EtOAc (1:1, R_f=0.5) to give 27 (87%) as a white solid: mp 77.5-78.5°C; ¹H-NMR (CDCl₃) δ 0.6 (d, 3H, J=7.2 Hz, CHCH₃), 0.9 (d, 3H, J=7.2 Hz, CHCH₃), 1.2 (t, 6H, J=7.2 Hz, CH₂CH₃), 1.3 (t, 6H, J=7.2 Hz, CH₂CH₃), 2.12-2.28 (m, 1H, CH(CH₃)₂), 2.36 (s, 3H, COCH₃), 3.81 (s, 3H, CH₃O-), 4.1-4.33 (m, 4H, 2 x CH₂CH₃), 4.8 (br, 1H, 2-H) and 6.9-7.8 (m, 3H, Ar-4,6,7H).

- 25 (b) 1-Acetyl-3-carboxy-2,3-dihydro-2-isopropyl-5-methoxyindole (28).

The acetylindole 27 was hydrolysed as described for the preparation of 11 to give 28 (90%) as an off-white foam (R_f=0.45, Me₂CO), which was used in the next step without further purification; ¹H-NMR (CDCl₃) δ 0.59 (d, 3H, J=7.2 Hz, CHCH₃), 0.9 (d, 3H,

$J=7.2$ Hz, CHCH_3), 2.15-2.22 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.2 (s, 3H, COCH_3), 3.72 (s, 3H, $\text{CH}_3\text{O}-$), 3.98 (br, 1H, 3- H), 4.62 (br, 1H, 2- H), 6.8-6.98 (m, 2H, Ar-4,7 H) and 7.78-7.85 (m, 1H, Ar-6 H).

(c) Methyl 1-acetyl-2,3-dihydro-2-isopropyl-5-methoxyindole-3-carboxylate (29).

- 5 In a procedure identical to that carried out on 11, compound 29 was prepared from compound 28 as a pale brown oil (93%); $^1\text{H-NMR}$ (CDCl_3) δ 0.71 (d, 3H, $J=7.2$ Hz, CHCH_3), 1.0 (d, 3H, $J=7.2$ Hz, CHCH_3), 2.1-2.2 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.32 (s, 3H, COCH_3), 3.69 (s, 3H, CO_2CH_3), 3.79 (s, 3H, $\text{CH}_3\text{O}-$), 3.8 (br, 1H, 3- H), 4.6 (br, 1H, 2- H), 6.8-6.98 (m, 2H, Ar-4, 7 H) and 7.78-7.85 (m, 1H, Ar-6 H).

10 **(d) Methyl 1-acetyl-2-isopropyl-5-methoxyindole-3-carboxylate (30).**

A solution of 29 (1.0 g, 4.0 mmol) was stirred under reflux with DDQ (0.96 g, 4.2 mmol) in toluene (12.5 mL) for 2 days. The DDQH_2 was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified on silica, eluting with hexane/ Me_2CO (3:1, $R_f=0.65$) to give 30 (0.44 g, 44%) as a pale red oil; $^1\text{H-NMR}$ (CDCl_3) δ 1.45 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.78 (s, 3H, COCH_3), 3.87 (s, 3H, CO_2CH_3), 3.96 (s, 3H, $\text{CH}_3\text{O}-$), 3.88-3.98 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 6.89 (dd, 1H, $J=2.7$ and 9 Hz, Ar-6 H), 7.44 (d, 1H, $J=9$ Hz, Ar-7 H) and 7.55 (d, 1H, $J=2.7$ Hz, Ar-4 H).

(e) Methyl 2-isopropyl-5-methoxyindole-3-carboxylate (31).

- 20 This compound was prepared from compound 30 as described for 14 (70%) as a pale red oil; $^1\text{H-NMR}$ (CDCl_3) δ 1.43 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 3.87 (s, 3H, CO_2CH_3), 3.92 (s, 3H, $\text{CH}_3\text{O}-$), 4.08-4.25 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 6.9 (dd, 2H, $J=2.7$ and 9 Hz, Ar-6 H), 7.1-7.6 (m, 2H, Ar-4,7 H) and 8.2 (br s, 1H, NH).

(f) Methyl 2-isopropyl-5-methoxy-1-methylindole-3-carboxylate (32).

- 25 In a procedure identical to that carried out on 14, compound 32 was prepared (80%) from compound 31 and purified on silica, eluting with EtOAc/hexane (1:2, $R_f=0.74$) as a pale yellow solid: mp 105-106°C; $^1\text{H-NMR}$ (CDCl_3)

δ 1.45 (d, 6H, $J=7.2\text{Hz}$, $\text{CH}(\text{CH}_3)_2$), 3.79 (s, 3H, $\text{CH}_3\text{N-}$), 3.88 (s, 3H, CO_2CH_3), 3.92 (s, 3H, $\text{CH}_3\text{O-}$), 4.22-4.45 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 6.9 (dd, 2H, $J=2.7$ and 9Hz , Ar-6H) and 7.1-7.64 (m, 1H, Ar-4,7H).

(g) Methyl 2-isopropyl-5-methoxy-1-methyl-4-nitroindole-3-carboxylate (33).

- 5 To a solution of **32** (8.0 g, 34.66 mmol) in AcOH (150 mL) cooled to 0°C , was added dropwise a cold (0°C) mixture of f.HNO_3 (27 mL) in AcOH (100 mL). The solution was stirred for 3 hours while allowing to reach room temperature, and then poured onto 300 g of crushed ice and the resulting yellow solid collected by suction filtration. The dried residue was purified on silica, eluting with EtOAc/hexane (1:2, $R_f=0.26$) to give
- 10 7.5 g (71%) of **33** as a yellow solid, recrystallised from EtOAc: mp $149-150.5^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.44 (d, 6H, $J=7.2\text{ Hz}$, $\text{CH}(\text{CH}_3)_2$), 3.78 (s, 3H, $\text{CH}_3\text{N-}$), 3.82 (s, 3H, CO_2CH_3), 3.91 (s, 3H, $\text{CH}_3\text{O-}$), 3.95-4.15 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 6.97 (d, 1H, $J=9\text{ Hz}$, Ar-6H) and 7.35 (d, 1H, $J=9\text{ Hz}$, Ar-7H).

(h) 2-Isopropyl-3-methoxycarbonyl-5-methoxy-1-methylindole-4,7-dione (35).

- 15 Compound **33** was reduced as described for **17** to give **34** (85%) and the crude material oxidized with Fremy's salt as described for **18**. After work-up the resulting orange precipitate was collected by suction filtration, washed with H_2O and dried in a vacuum oven at 45°C to afford **35** as an orange solid (75%), recrystallized from EtOAc: mp $192-194^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.34 (d, 6H, $J=7.2\text{ Hz}$, $\text{CH}(\text{CH}_3)_2$), 3.12-3.28
- 20 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.81 (s, 3H, $\text{CH}_3\text{N-}$), 3.90 (s, 3H, CO_2CH_3), 3.97 (s, 3H, $\text{CH}_3\text{O-}$) and 5.64 (s, 1H, 6-H). Anal. C; 62.31, H; 5.82, N; 4.90% Calc. ($\text{C}_{15}\text{H}_{17}\text{NO}_5$) C; 61.86, H; 5.84, N; 4.81%.

Example 11: 3-Hydroxymethyl-2-isopropyl-5-methoxy-1-methylindole-4,7-dione (36) by reaction (xviii) of Figure 2.

- 25 To a solution of **35** (0.5 g, 1.7 mmol) in CHCl_3 (50 mL) and EtOH (18 mL) was added a solution of $\text{Na}_2\text{S}_2\text{O}_4$ (3.5 g, 20 mmol) in H_2O (22 mL). The solution was stirred at room temperature for 1 hour and the organic layer separated, washed with sat. NaCl

(50 mL), dried and evaporated. The crude hydroquinone was dried over 18 hours *in vacuo* and then dissolved in anhydrous THF (5 mL) under argon and added to a solution of LiAlH_4 (12 mL of a 1.0 M solution in THF) dropwise at room temperature and under argon. The solution was then stirred for 1 hour at 30°C, cooled to 0°C, and

5 H_2O (15 mL) added dropwise, followed by a solution of FeCl_3 (12 mL, 1.0 M (0.1 M HCl)) added at 0°C. The solution was stirred for 10 minutes at 0°C and then EtOAc (150 mL) and H_2O (150 mL) added. The aqueous layer was extracted with EtOAc (5 x 50 mL) and the organic phase washed with sat. NaCl (250 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc/hexane,

10 (1:1, $R_f=0.22$) to give, after recrystallization from EtOAc, 36 as an orange solid (140 mg, 31 %): mp 160-161°C; $^1\text{H-NMR}$ (CDCl_3) δ 1.37 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.82 (s, 3H, $\text{CH}_3\text{N-}$), 3.97 (s, 3H, $\text{CH}_3\text{O-}$), 4.71 (br, 2H, CH_2OH) and 5.64 (s, 1H, 6-*H*). Anal. C; 63.42, H; 6.50, N; 5.24% Calc. ($\text{C}_{14}\text{H}_{17}\text{NO}_4$) C; 63.88, H; 6.46, N; 5.32%.

15 **Example 12: 5-(Aziridin-1-yl)-3-hydroxymethyl-2-isopropyl-1-methylindole-4,7-dione (37) by reaction (xx) of Figure 2.**

A solution of 36 (50 mg, 0.19 mmol) in 1(H)-aziridine (0.5 mL, ca. 11.7 mmol, CAUTION!) was stirred for 0.5 hour at room temperature and then evaporated to dryness and the residue purified on silica, eluting with EtOAc ($R_f=0.55$) to give, after

20 recrystallization from EtOAc, 37 (42 mg, 81%) as a red solid: mp 144-145°C; $^1\text{H-NMR}$ (CDCl_3) δ 1.37 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.18 (s, 4H, 2 x azir- CH_2), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.95 (s, 3H, $\text{CH}_3\text{N-}$), 4.68 (br s, 1H, CH_2OH), 4.76 (br, 2H, CH_2OH) and 5.76 (s, 1H, 6-*H*). Anal. C; 65.74, H; 6.64, N; 9.91% Calc. ($\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_3$) C; 65.69, H; 6.57, N; 10.22%.

25 **Example 13: 3-Hydroxymethyl-2-isopropyl-5-(2-methylaziridin-1-yl)-1-methylindole-4,7-dione (38) by reaction (xx) of Figure 2.**

Compound 36 (0.1 g, 0.38 mmol) was dissolved and stirred in freshly distilled 2-methylaziridine (3 mL, ca. .50 mmol) for 2.5 hours. The solution was evaporated

in vacuo and the residue redissolved in EtOAc, evaporated and purified on silica (eluting with EtOAc) to afford a red glass (38, 85 mg, 78%): $^1\text{H-NMR}$ (CDCl_3) δ 1.37 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 1.42 (d, 3H, $J=4.5$ Hz, azir- CH_3), 2.01-2.15 (m, 3H, azir- CHCH_2), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.95 (s, 3H, $\text{CH}_3\text{N-}$), 4.68 (br s, 1H, CH_2OH), 4.76 (br, 2H, CH_2OH) and 5.76 (s, 1H, 6- H). Anal. C; 67.12, H; 6.04, N; 9.57% Calc. ($\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_3$) C; 66.67, H; 6.94, N; 9.72%.

Example 14: Preparation of 2-isopropyl-5-methoxy-1-methyl-3-[(carbamoyloxy)-methyl]-indole-4,7-dione (40) by reactions (xxi) and (xxii) of Figure 2.

(a) 2-Isopropyl-5-methoxy-1-methyl-3-[[phenoxycarbonyloxy]methyl] indole-4,7-dione (39).

To a solution of 36 (0.55 g, 2.1 mmol) in anhydrous pyridine (25 mL) at 0°C , was added dropwise phenylchloroformate (0.5 g, 3.2 mmol) and the solution then allowed to reach room temperature and stirred for 1 hour and then a further 0.25 g (1.6 mmol) of phenylchloroformate was added. After a further 1 hour, H_2O (200 mL) was added and the solution was extracted with EtOAc (4 x 50 mL) and washed with H_2O (100 mL) and sat. NaCl (100 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc ($R_f=0.8$) to give 39 (0.6 g, 75%) as an orange solid: mp $126-127^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.37 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.79 (s, 3H, $\text{CH}_3\text{N-}$), 4.01 (s, 3H, $\text{CH}_3\text{O-}$), 5.27 (s, 2H, CH_2OCOPh), 5.51 (s, 1H, 6- H) and 7.15-7.3 (m, 5H, Ar).

(b) 2-Isopropyl-5-methoxy-1-methyl-3-[(carbamoyloxy)methyl] indole-4,7-dione (40).

The phenylcarbonate 39 (0.1 g, 0.26 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and the solution cooled to -78°C . The solution was then saturated with NH_3 and stirred at -78°C until reaction was complete (ca. 2.5 hours). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (50 mL) and washed with H_2O (2 x 50 mL) and sat. NaCl (25 mL), dried and evaporated, and the residue recrystallized from EtOAc to afford

55 mg (69%) of 40 as an orange solid: mp 244-246°C (dec.); ¹H-NMR (CDCl₃) δ 1.37 (d, 6H, J=7.2 Hz, CH(CH₃)₂), 3.1-3.28 (m, 1H, CH(CH₃)₂), 3.78 (s, 3H, CH₃N-), 3.99 (s, 3H, CH₃O-), 4.79 (br s, 2H, NH₂), 5.3 (s, 2H, CH₂OCONH₂) and 5.62 (s, 1H, 6-H). Anal. C; 58.67, H; 5.76, N; 9.02%, Calc. (C₁₅H₁₈N₂O₅) C; 58.82, H; 5.88, N; 9.15%.

Example 15: 5-(Aziridin-1-yl)-2-isopropyl-1-methyl-3-[(carbamoyloxy)methyl] indole-4,7-dione (41) by reaction (xxiii) of Figure 2.

The carbamate 40 (0.1 g, 0.33 mmol) was stirred at room temperature in 1(H)-aziridine (2 mL, CAUTION!) for 25 minutes, evaporated and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume and the resulting red precipitate filtered and washed well with cold EtOAc to afford 41 (55 mg, 53%) as a red solid: mp 230-233°C(dec.); ¹H-NMR ((CD₃)₂SO) δ 1.37 (d, 6H, J=7.2 Hz, CH(CH₃)₂), 2.18 (s, 4H, 2 x azir-CH₂), 3.1-3.28 (m, 1H, CH(CH₃)₂), 3.93 (s, 3H, CH₃N-), 5.06 (s, 2H, CH₂OCONH₂), 5.78 (s, 1H, 6-H) and 6.42 (br s, 2H, NH₂). Anal. C; 60.25, H; 6.04, N; 13.30%, Calc. (C₁₆H₁₉N₃O₄) C; 60.57, H; 5.99, N; 13.25%.

Example 16: 2-Isopropyl-1-methyl-5-(2-methylaziridin-1-yl)-3-[(carbamoyloxy)methyl] indole-4,7-dione (42) by reaction (xxiii) of Figure 2.

The carbamate 40 (0.1 g, 0.32 mmol) was stirred at room temperature in 2-methylaziridine (1.5 mL) for 2.5 hours, evaporated and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume and the resulting red precipitate filtered and washed well with cold EtOAc, and then recrystallized from EtOAc to afford 42 (55 mg, 52%) as a red solid: mp 204-205°C(dec.); ¹H-NMR ((CD₃)₂SO) δ 1.37 (d, 6H, J=7.2 Hz, CH(CH₃)₂), 1.42 (d, 3H, J=5.4 Hz, azir-CH₃), 1.98-2.05 (m, 3H, azir-CHCH₂), 3.1-3.28 (m, 1H, CH(CH₃)₂), 3.93 (s, 3H, CH₃N-), 5.06 (s, 2H, CH₂OCONH₂), 5.76 (s, 1H, 6-H) and 6.41 (br s, 2H, NH₂). Anal. C; 61.52, H; 6.36, N; 12.90%, Calc. (C₁₇H₂₁N₃O₄) C; 61.63, H; 6.34, N; 12.69%.

Example 17: 1,3-Dimethyl-2-isopropyl-5-methoxyindole-4,7-dione (43).

To a solution of 35 (0.5 g, 1.7 mmol) in CHCl_3 (50 mL) and EtOH (18 mL) was added a solution of $\text{Na}_2\text{S}_2\text{O}_4$ (3.5 g, 20 mmol) in H_2O (22 mL). The solution was stirred at room temperature for 1 hour and the organic layer separated, washed with sat. NaCl (50 mL), dried and evaporated. The crude hydroquinone was dried over 18 hours *in vacuo* and then dissolved in anhydrous THF (5 mL) under argon and added to a solution of DIBAL-H (10 mL of a 1.5 M solution in toluene) dropwise at -30°C and under argon. The solution was then stirred for 18 hours at 4°C , cooled to 0°C , and H_2O (15 mL) added dropwise, followed by a solution of FeCl_3 (12 mL, 1.0 M (0.1 M HCl)) added at 0°C . The solution was stirred for 10 minutes at 0°C and then EtOAc (150 mL) and H_2O (150 mL) added. The aqueous layer was extracted with EtOAc (5 x 50 mL) and the organic phase washed with sat. NaCl (250 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc/hexane, (1: 2, $R_f=0.44$) to give, after recrystallization from EtOAc, 43 as an orange solid (48 mg, 11 %): mp $168-169^\circ\text{C}$;
 $^1\text{H-NMR}$ (CDCl_3) δ 1.35 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.38 (s, 3H, 3- CH_3), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.78 (s, 3H, $\text{CH}_3\text{N-}$), 3.95 (s, 3H, $\text{CH}_3\text{O-}$) and 5.57 (s, 1H, 6- H).

Example 18: 5-(Aziridin-1-yl)-1,3-dimethyl-2-isopropylindole-4,7-dione (44).

A solution of 43 (30 mg, 0.12 mmol) in 1(H)-aziridine (0.5 mL, ca. 11.7 mmol, CAUTION!) was stirred for 0.5 hour at room temperature and then evaporated to dryness and the residue purified on silica, eluting with EtOAc/hexane (1:2, $R_f=0.32$) to give, after recrystallization from EtOAc, 44 (5.5 mg, 18%) as a red solid: mp $110-112^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.35 (d, 6H, $J=7.2$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.15 (s, 4H, 2 x azir- CH_2), 2.38 (s, 3H, 3- CH_3), 3.1-3.28 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.94 (s, 3H, $\text{CH}_3\text{N-}$) and 5.72 (s, 1H, 6- H).

Example 19: 2-Cyclohexyl-3-hydroxymethyl-5-methoxy-1-methylindole-4,7-dione (45).

The corresponding 3-methoxycarbonyl precursor compound was prepared as described for 18 and 35 but using cyclohexylcarboxaldehyde in the in-line cyclisation step and the subsequent oxidation step with DDQ required a much prolonged reaction time of 12h. The crude 4-amino compound was again oxidized with Fremy's salt as described for 18. After work-up the resulting orange precipitate was collected by suction filtration, washed with H₂O and dried in a vacuum oven at 45°C to give 2-cyclohexyl-3-methoxycarbonyl-5-methoxy-1-methylindole-4,7-dione as an orange solid (75%), recrystallized from EtOAc: mp 190-192°C; ¹H-NMR (CDCl₃) δ 1.27 (m, 6H, 6 x cyclohexyl-H), 1.83 (m, 5H, 5 x cyclohexyl-H), 3.79 (s, 3H, CH₃N-), 3.91 (s, 3H, CO₂CH₃), 3.96 (s, 3H, CH₃O-) and 5.63 (s, 1H, 6-H). This compound was then reduced with LiAlH₄ as described for 36 to give 45 as an orange solid (59 %): mp 214-215°C; ¹H-NMR (CDCl₃) δ 1.25 (m, 6H, 6 x cyclohexyl-H), 1.78 (m, 5H, 5 x cyclohexyl-H), 3.81 (s, 3H, CH₃N-), 3.97 (s, 3H, CH₃O-), 4.76 (s, 2H, CH₂OH) and 5.63 (s, 1H, 6-H). Anal. C; 67.54, H; 6.82, N; 4.67% Calc. (C₁₇H₂₁NO₄) C; 67.31, H; 6.98, N; 4.62%.

Example 20: 5-(Aziridin-1-yl)-2-cyclohexyl-3-hydroxymethyl-1-methylindole-4,7-dione (46).

A solution of 45 (30mg, 0.1mmol) in freshly distilled 1(H)-aziridine (0.5mL, ca. 11.7mmol, CAUTION !) was stirred for 0.5h at room temperature and then evaporated to dryness and the residue purified on silica, eluting with EtOAc/hexane. (1:1, R_f=0.3) to give, after recrystallization from EtOAc, 46 (22mg, 70%) as a red solid: mp 128-130°C; ¹H-NMR (CDCl₃) δ 1.26 (m, 6H, 6 x cyclohexyl-H), 1.78 (m, 5H, 5 x cyclohexyl-H), 2.18 (s, 4H, 2 x aziridine-CH₂), 3.95 (s, 3H, CH₃N-), 4.76 (s, 2H, CH₂OH) and 5.77 (s, 1H, 6-H). Anal. C; 63.72, H; 6.97, N; 8.22% Calc. (C₁₈H₂₂N₂O₃·1.5H₂O) C; 63.34, H; 7.33, N; 8.21%.

Example 21: Preparation of 5-Methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione (52) by reactions (i) to (vi) of Figure 3.

(a) 5-Methoxy-1,2-dimethylindole (47).

5-Methoxy-2-methylindole (10 g, 0.062 mol) was added gradually and under dry argon to a stirred suspension of NaH (2.73 g of a 60% dispersion, 0.068 mol) in DMF (150 mL). The suspension was heated at 45°C for 10 minutes, cooled to room temperature, and MeI (33 mL, 0.23 mol) added over 5 minutes. The solution was then heated at 60°C for 1 hour, cooled and poured onto cold (0°C) NaHSO₄ (aq., 10%, 150 mL) and extracted with EtOAc (3 x 100 mL), dried and evaporated. The residue was purified on silica, eluting with 3% EtOAc/hexane (R_f=0.5) to give 4.5 g (41%) of 45 as a pale brown solid: mp 73-74°C; ¹H-NMR (CDCl₃) δ 2.37 (s, 3H, 2-CH₃), 3.59 (s, 3H, CH₃N-), 3.82 (s, 3H, CH₃O-), 6.16 (s, 1H, 3-H) and 6.9-7.28 (m, 3H, Ar-4,6,7H).

(b) 5-Methoxy-1,2-dimethylindole-3-carboxaldehyde (48).

15 N-methylformanilide (0.95 g, 7.04 mmol) and POCl₃ (1.08 g, 7.05 mmol) were stirred at room temperature until the yellow solid chloroimmonium Vilsmeier compound formed. The yellow solid was then added to a solution of 47 (0.7 g, 4 mmol) in 1,2-dichloroethane (15 mL) and the solution heated under reflux for 1.5 hours, cooled, and NaOAc (1.0 M, 50 mL) added. The solution was extracted with EtOAc (4 x 100 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, R_f=0.5 (EtOAc)) to give 0.35 g (43%) of 48 as an off-white solid: mp 108-110°C; ¹H-NMR (CDCl₃) δ 2.62 (s, 3H, 2-CH₃), 3.63 (s, 3H, CH₃N-), 3.89 (s, 3H, CH₃O), 6.94-7.2 (m, 2H, Ar-6,7H), 7.8 (d, 1H, J=2 Hz, Ar-4H) and 10.1 (s, 1H, CHO).

25 (c) 5-Methoxy-1,2-dimethyl-4-nitroindole-3-carboxaldehyde (49) by reaction (iii). Compound 48 (6.0 g, 0.03 mol) was dissolved in AcOH (480 mL) and cooled to 5°C and f.HNO₃ (18 mL) in AcOH (72 mL) added dropwise with stirring over 5 minutes. The solution temperature was allowed to rise to 20°C over 18 hours and the mixture

poured on to crushed ice (500 g) and the yellow precipitate collected by suction filtration and dried *in vacuo* at 50°C. The yellow solid was purified on silica, eluting with EtOAc/hexane (2:1, R_f=0.42 (EtOAc)) to give 4.37 g (59%) of 49 as a pale yellow solid: mp 236-238°C(dec.); ¹H-NMR ((CD₃)₂SO) δ 2.71 (s, 3H, 2-CH₃), 3.76 (s, 3H, CH₃N-), 3.89 (s, 3H, CH₃O), 7.25 (d, 1H, J=9 Hz, Ar-7H), 7.75 (d, 1H, J=9 Hz, Ar-6H) and 9.9 (s, 1H, CHO).

(d) **4-Amino-5-methoxy-1,2-dimethylindole-3-carboxaldehyde (50)** by reaction (iv). Nitro-compound 49 (0.18 g, 0.74 mmol) was dissolved in EtOH (15 mL) and powdered tin (0.45 g, 3.8 mmol) added, followed by HCl (3.0 M, 5.6 mL) and the solution heated under gentle reflux for 1 hour. Water (50 mL) was added and the solution neutralized with NaHCO₃ (aq.) and then extracted with CHCl₃ (3 x 100 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, R_f=0.57 (EtOAc)) to give 0.1 g (62%) of 50 as a pale yellow solid: mp 152-153°C (dec.); ¹H-NMR ((CD₃)₂SO) δ 2.61 (s, 3H, 2-CH₃), 3.59 (s, 3H, CH₃N-), 3.75 (s, 3H, CH₃O), 6.0 (br s, 2H, NH₂), 6.56 (d, 1H, J=9 Hz, Ar-7H), 6.85 (d, 1H, J=9 Hz, Ar-6H) and 9.71 (s, 1H, CHO).

(e) **5-Methoxy-1,2-dimethyl-4,7-dioxoindole-3-carboxaldehyde (51)**.

To a solution of 50 (0.074 g, 0.34 mmol) in Me₂CO (14 mL) was added a solution of Fremy's salt (0.45 g, 1.68 mmol) in NaH₂PO₄/Na₂HPO₄ buffer (14 mL, 0.3 M, pH 6.0) and the solution stirred at room temperature for 1 hour. The solution was evaporated at 30°C to remove most of the Me₂CO and the resulting orange precipitate collected by suction filtration and washed well with H₂O and cold MeOH, to give 51 (0.06 g, 75%): mp 239-242°C; ¹H-NMR ((CD₃)₂SO) δ 2.5 (s, 3H, 2-CH₃), 3.82 (s, 3H, CH₃N-), 3.88 (s, 3H, CH₃O), 5.89 (s, 1H, 6-H) and 10.37 (s, 1H, CHO).

(f) **5-Methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione (52)** by reaction (vi).

To a suspension of 51 (0.25 g, 0.94 mmol) in MeOH (100 mL, degassed by boiling in under argon *in vacuo*) was added NaBH₄ (0.36 g, 9.7 mmol) while maintaining a dry

argon atmosphere. The solution was stirred at room temperature for 1 hour and the solution aerated prior to the addition of H₂O (40 mL) and the solution extracted with CH₂Cl₂ (3 x 100 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc (R_f=0.4) to afford **52** (0.1 g, 33%) as an orange solid, recrystallized from EtOAc: mp 215-216°C; ¹H-NMR (CDCl₃) δ 2.21 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N-), 3.86 (s, 3H, CH₃O), 4.65 (br d, 2H, J=7.2 Hz, CH₂OH) and 5.61 (s, 1H, 6-H)

Example 22: 5-(Aziridin-1-yl)-3-hydroxymethyl-1,2-dimethylindole-4,7-dione (53) via reaction (vii) of Figure 4.

A solution of **52** (235 mg, 1.0 mmol) in freshly redistilled 1(H)-aziridine (1.5 mL, ca. 35 mmol, CAUTION!) was stirred for 0.5 hour at room temperature and evaporated *in vacuo*. The residue was redissolved in EtOAc, condensed by 75%, and the precipitate collected and washed with cold EtOAc, to give 225 mg (91%) of **53** as a dark red solid: mp 173-174°C (dec.); ¹H-NMR (CDCl₃) δ 2.19 (s, 4H, 2 x azir-CH₂), 2.22 (s, 3H, 2-CH₃), 3.86 (s, 3H, CH₃N-), 3.95 (br, 1H, CH₂OH), 4.65 (br d, 2H, CH₂OH) and 5.76 (s, 1H, 6-H). Anal. C; 63.08, H; 5.62, N; 11.07% Calc. (C₁₃H₁₄N₂O₃) C; 63.41, H; 5.69, N; 11.38%.

Example 23 : 3-Hydroxymethyl-5-(2-methylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (54) by reaction (vii) of Figure 4.

A solution of **52** (235 mg, 1.0 mmol) in 2-methylaziridine (2 mL, ca. mmol) was stirred at room temperature for 4 hours and worked up as described for **53** to give **54** (195 mg, 75%) as a dark red solid: mp 120-122°C; ¹H-NMR (CDCl₃) δ 1.42 (d, 3H, J=5.4 Hz, azir-CH₃), 2.08-2.21 (m, 3H, azir-CHCH₂), 2.22 (s, 3H, 2-CH₃), 3.89 (s, 3H, CH₃N-), 4.61 (br, 1H, CH₂OH), 4.63 (br, 2H, CH₂OH) and 5.75 (s, 1H, 6-H). Anal. C; 65.09, H; 5.79, N; 10.72% Calc. (C₁₄H₁₆N₂O₃) C; 64.61, H; 6.15, N; 10.77%.

Example 24: 3-Hydroxymethyl-5-(cis-2,3-dimethylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (55) by reaction (vii) of Figure 4.

A solution of 52 (100 mg, 0.43 mmol) in cis-2,3-dimethylaziridine (2 mL) was stirred for 1 hour at room temperature and then evaporated *in vacuo* at 30°C. The residue was
 5 purified on silica, eluting with EtOAc (Rf=0.6), to give 55 (65 mg, 55%) after recrystallization from EtOAc: mp 132-135°C; ¹H-NMR (CDCl₃) δ 1.38 (d, 6H, J=5.4Hz, 2 x azir-CH₃), 2.15-2.21 (m, 2H, 2 x azir-CH), 2.22 (s, 3H, 2-CH₃), 3.85 (s, 3H, CH₃N-), 4.24 (br, 1H, CH₂OH), 4.63 (br, 2H, CH₂OH) and 5.73 (s, 1H, 6-H).
 Anal. C; 65.93, H; 6.50, N; 10.12% Calc. (C₁₅H₁₈N₂O₃) C; 65.69, H; 6.57,
 10 N; 10.22%.

Example 25: 3-Hydroxymethyl-5-(2,2-dimethylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (56) by reaction (viii) of Figure 4.

A solution of 52 (100 mg, 0.43 mmol) in 2,2-dimethylaziridine (2 mL) was stirred at 95°C for 5 hours. After this time (reaction does not go to completion) the solution was
 15 cooled and evaporated *in vacuo*, and the residue redissolved in EtOAc and evaporated to precipitate 56 as a dark red solid, which was unstable on silica and in solution: mp 138-141°C; ¹H-NMR (CDCl₃) δ 1.32 (s, 6H, 2 x azir-CH₃), 2.07 (s, 2H, azir-CH₂), 2.22 (s, 3H, 2-CH₃), 3.87 (s, 3H, CH₃N-), 4.25 (br, 1H, CH₂OH), 4.86 (br, 2H, CH₂OH) and 5.68 (s, 1H, 6-H). Anal. C; 62.92, H; 6.88, N; 9.58%
 20 Calc. (C₁₅H₁₈N₂O₃.2/3H₂O) C; 62.94, H; 6.76, N; 9.79%.

Example 26: 3-Hydroxymethyl-5-(2,2-dimethyl-2-hydroxyethylamino)-1,2-dimethylindole-4,7-dione (57) by reaction (viii) of Figure 4.

Compound 56 (25 mg, mmol) was dissolved in 5 mL H₂O with warming to 45°C and then evaporated *in vacuo* at 50°C. The residue was purified on silica, eluting with
 25 EtOAc (Rf=0.35) to give 57, after recrystallization from EtOAc: mp 188-190°C; ¹H-NMR ((CD₃)₂SO) δ 1.14 (s, 6H, 2 x CH₃), 2.21 (s, 3H, 2-CH₃), 3.02 (d, 2H, J=7.2 Hz, NHCH₂C(CH₃)₂), 3.84 (s, 3H, CH₃N-), 4.58 (br, 2H, CH₂OH), 4.65 (br, 1H, CH₂OH), 5.14 (s, 1H, 6-H) and 6.63 (br t, 1H, NH). Anal. C; 60.62, H; 6.96, N; 9.34%
 Calc. (C₁₅H₂₀N₂O₄.1/3H₂O) C; 60.40, H; 6.94, N; 9.39%.

Example 27: Preparation of 3-Hydroxymethyl-5-(2-methylaziridin-1-yl)-1-methyl-indole-4,7-dione (62) by reactions (I) to (v) of Figure 5.

(a) 5-Methoxy-1-methylindole-3-carboxaldehyde (58).

5-methoxyindole-3-carboxaldehyde (2.0 g, 11.4 mmol) was added portionwise to a suspension of NaH (0.55 g, 13.7 mmol) in DMF (50 mL) with stirring. The suspension was stirred for 0.5 hour, and MeI (1.94 g, 13.7 mmol) added and the mixture stirred for 1 hour at room temperature. The reaction mixture was then poured on to NaHCO₃ (10%, 300 mL) and extracted with EtOAc (4 x 75 mL), washed with NaHCO₃ (10%, 3 x 50 mL), sat. NaCl (3 x 100 mL), dried and evaporated *in vacuo* to give 58 (1.70 g, 79%) as a white solid: mp 132-133°C; ¹H-NMR (CDCl₃) δ 3.81 (s, 3H, CH₃N-), 3.89 (s, 3H, CH₃O-), 7.08 (dd, 1H, J=2 and 9 Hz, Ar-6H), 7.31 (d, 1H, J=9 Hz, Ar-7H), 7.59 (s, 1H, 2-H), 7.8 (d, 1H, J=2 Hz, Ar-4H) and 9.93 (s, 1H, CHO).

(b) 5-Methoxy-1-methyl-4-nitroindole-3-carboxaldehyde (59).

To a solution of 56 (1.50 g, 7.94 mmol) dissolved in AcOH (150 mL) was added a mixture of c.HNO₃ (4.5 mL) in AcOH (25 mL) dropwise at 0°C over 3 hours. After addition, the mixture was stirred at room temperature for 16 hours, and then added to crushed ice (75 g), filtered and washed with H₂O (5 x 100 mL) and dried to give 57 (1.56 g, 84%) as a pale yellow solid: mp 197-198°C; ¹H-NMR ((CD₃)₂SO) δ 3.94 (s, 6H, CH₃O- and CH₃N-), 7.32 (d, 1H, J=9 Hz, Ar-7H), 7.72 (d, 1H, J=9 Hz, Ar-6H), 8.27 (s, 1H, 2-H) and 9.75 (s, 1H, CHO).

(c) 4-Amino-5-methoxy-1-methylindole-3-carboxaldehyde (60).

To a suspension of 59 (1.0 g, 4.27 mmol) in EtOH (150 mL) was added tin (4.43 g, 37 mmol) followed by HCl (3.0 M, 60 mL). The mixture was stirred at room temperature for 2 hours and decanted. The solution was added portionwise to sat. NaHCO₃ (aq., 300 mL) and extracted with EtOAc (3 x 100 mL). The organic layer was separated, washed with sat. NaHCO₃ (aq., 2 x 175 mL), sat. NaCl (3 x 75 mL), dried and evaporated *in vacuo* to give 60 (0.79 g, 91%) as a dark yellow solid which was used in the next step without further purification; R_f=0.64 (EtOAc);

$^1\text{H-NMR}$ (CDCl_3) δ 3.75 (s, 3H, $\text{CH}_3\text{N-}$), 3.88 (s, 3H, $\text{CH}_3\text{O-}$), 5.79 (br s, 2H, NH_2), 6.56 (d, 1H, $J=9$ Hz, Ar-7H), 7.53 (d, 1H, $J=9$ Hz, Ar-6H), 7.64 (s, 1H, 2-H) and 9.60 (s, 1H, CHO).

(d) 5-methoxy-1-methyl-4,7-dioxoindole-3-carboxaldehyde (61).

- 5 To **60** (0.75 g, 3.68 mmol) dissolved in Me_2CO (75 mL) was added Fremy's salt (4.0 g, 14.9 mmol) in H_2O (20 mL) followed by a solution of $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer (0.3 M, pH 6, 20 mL). The mixture was stirred for 0.75 hour, excess Me_2CO removed and the product filtered and washed with H_2O (50 mL), dried, and recrystallized from EtOAc to give **61** (0.61 g, 76%) as a yellow solid: mp 188-190°C; $^1\text{H-NMR}$ (CDCl_3)
- 10 δ 3.87 (s, 3H, $\text{CH}_3\text{N-}$), 4.02 (s, 3H, $\text{CH}_3\text{O-}$), 5.78 (s, 1H, 6-H), 7.44 (s, 1H, 2-H) and 10.54 (s, 1H, CHO).

(e) 3-Hydroxymethyl-5-methoxy-1-methylindole-4,7-dione (62).

- To a solution of **61** (0.5 g, 2.28 mmol) in anhydrous MeOH (300 mL) was added NaBH_4 (0.65 g, 17 mmol). The solution was degassed with argon and stirred
- 15 for 2 hours under argon and then evaporated *in vacuo* to give a solid which was diluted with CH_2Cl_2 (300 mL) and washed with H_2O (2 x 100 mL), sat. NaCl (100 mL) and condensed to give **62** as an orange solid (0.2 g, 40%) after recrystallization from EtOAc: mp 185-186°C; $^1\text{H-NMR}$ (CDCl_3) δ 3.85 (s, 3H, $\text{CH}_3\text{N-}$), 3.94 (s, 3H, $\text{CH}_3\text{O-}$), 4.25-4.29 (m, 2H, CH_2OH), 5.73 (s, 1H, 6-H) and 6.88 (s, 1H, 2-H).
- 20 Anal. C; 59.84, H; 4.79, N; 6.30% Calc. ($\text{C}_{11}\text{H}_{11}\text{NO}_4$) C; 59.73, H; 4.98, N; 6.33%.

Example 28: Preparation of 5-(Aziridin-1-yl)-3-hydroxymethyl-1-methylindole-4,7-dione (63) by reaction (vi) of Figure 5.

- A solution of **62** (200 mg, 0.9 mmol) in 1(H)-aziridine (1.5 mL, ca. 35 mmol, CAUTION!) was stirred at room temperature for 1.5 hours. Excess aziridine was
- 25 removed *in vacuo*, and the product was recrystallized from EtOAc to give **63** (130 mg, 62%) as an orange solid: mp 169-171°C; $^1\text{H-NMR}$ (CDCl_3) δ 2.22 (s, 4H, 2 x aziridine- CH_2), 3.91 (s, 3H, $\text{CH}_3\text{N-}$), 4.64 (s, 2H, CH_2OH), 5.81 (s, 1H, 6-H),

and 6.69 (s, 1H, 2-*H*). Anal. C; 61.47, H; 5.14, N; 12.22% Calc. (C₁₂H₁₂N₂O₃)
C; 62.07, H; 5.17, N; 12.07%.

Example 29: Preparation of 3-Hydroxymethyl-5-(2-methylaziridin-1-yl)-1-methyl-indole-4,7-dione (64) by reaction (vi) of Figure 5.

- 5 A solution of **62** (200 mg, 0.9 mmol) in 2-methylaziridine (1 mL) was stirred at room temperature for 2.5 hours. Excess 2-methylaziridine was removed *in vacuo*, and the product was purified on silica, eluting with EtOAc (R_f=0.6) to give **64** (120 mg, 54%) as a red solid recrystallized from EtOAc: mp 89-90°C; ¹H-NMR (CDCl₃) δ 1.47 (d, 3H, *J*=4.5 Hz, azir-CH₃), 2.04-2.2 (m, 3H, azir-CHCH₂), 3.91 (s, 3H, N-CH₃), 4.67 (m, 2H, CH₂OH), 5.79 (s, 1H, 6-*H*) and 6.70 (s, 1H, 2-*H*).
- 10

Example 30: Preparation of 5-methoxy-1-methyl-3-[(carbamoyloxy)methyl] indole-4,7-dione (65) by reaction (vii) of Figure 5.

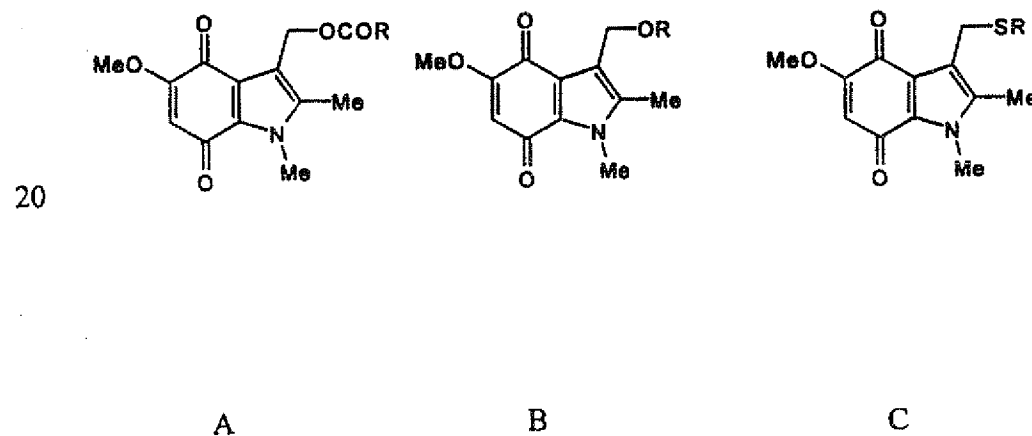
- To a solution of **62** (0.1 g, 0.45 mmol) in anhydrous pyridine (5 mL) at 0°C, was added dropwise phenylchloroformate (0.1 g, 0.64 mmol) and the solution then allowed to reach room temperature and stirred for 2 hours. The solution was then extracted with CH₂Cl₂ (30 mL) and washed with H₂O (35 mL) and sat. NaCl (35 mL), dried and evaporated. The residue was purified on silica, eluting with EtOAc (R_f=0.8) to give an orange solid of 5-methoxy-1-methyl-3-[[[phenoxycarbonyl]oxy]methyl] indole: mp 85-86°C; ¹H-NMR ((CD₃)₂SO/CDCl₃) δ 3.79 (s, 3H, CH₃N-), 3.91 (s, 3H, CH₃O-), 5.43 (s, 2H, CH₂OCOPh), 5.67 (s, 1H, 6-*H*), 6.89 (s, 1H, 2-*H*) and 7.15-7.3 (m, 5H, Ar). This material (0.1 g, 0.3 mmol) was dissolved in anhydrous CH₂Cl₂ (18 mL) and the solution cooled to -78°C. The solution was then saturated with NH₃ and stirred at -78°C until reaction was complete (0.75 hour). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was
- 15
- 20 redissolved in CH₂Cl₂ (100 mL) and washed with H₂O (2 x 100 mL) and sat. NaCl (50 mL), dried and evaporated, and the residue recrystallized from EtOAc to afford 75 mg (98%) of **65** as an orange solid: mp 231-234°C (dec.); ¹H-NMR ((CD₃)₂SO/CDCl₃) δ 3.78 (s, 3H, CH₃N-), 3.88 (s, 3H, CH₃O-),
- 25

5.04 (s, 2H, CH₂OCONH₂), 5.77 (s, 1H, 6-*H*), 6.42 (br s, 2H, NH₂) and 7.09 (s, 1H, 2-*H*). Anal. C; 54.98, H; 4.56, N; 10.18%, Calc. (C₁₂H₁₂N₂O₅) C; 54.55, H; 4.55, N; 10.61%.

Example 31: Preparation of 5-(Aziridin-1-yl)-1-methyl-3-[(carbamoyloxy)methyl]indole-4,7-dione (66) by reaction (viii) of Figure 5.

5 **Compound 65 (50 mg, 0.2 mmol) was stirred for 0.5 hour in 1(*H*)-aziridine**
 (ca. 35 mmol), evaporated *in vacuo* and redissolved in EtOAc. The solution was
 evaporated again and the residue recrystallized from EtOAc to afford 35 mg (70%) of
 66 as a red solid: mp 195-198°C (dec.); ¹H-NMR ((CD₃)₂SO) δ 2.18 (s, 4H, 2 x azir-
 10 CH₂), 3.90 (s, 3H, CH₃N-), 5.23 (s, 2H, CH₂OCONH₂), 5.49 (br s, 2H, NH₂)
 5.76 (s, 1H, 6-*H*) and 6.86 (s, 1H, 2-*H*). Anal. C; 56.70, H; 4.94, N; 15.35%,
 Calc. (C₁₃H₁₃N₃O₄) C; 56.73, H; 4.73, N; 15.27%.

Further compounds were prepared in order to investigate the effect of different leaving
 groups at the R¹ position in Formula I; these compounds being divided into three
 15 formula options A, B and C and the corresponding chloromethyl compound. By keeping
 the groups R, R² and R³ methyl and R⁴ hydrogen, as in Examples 32 to 44 and 46
 to 49, bioassay could be used to evaluate the effect of different leaving groups.
 Example 45 describes a preferred compound where R² is cyclopropyl.



Example 32: Preparation of 3-Chloromethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (67)

5-Methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione (52, 500mg, 1.97mmol) was stirred at room temperature with 5mL SOCl_2 for 0.5h. The solution was then
5 evaporated *in vacuo*, redissolved in EtOAc (25mL) and evaporated to dryness.

This procedure was repeated twice and the crude orange solid (0.43 g, 85%) of 3-chloromethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (mp 204-205°C (dec.)) was used in the next step without further treatment. A small sample was recrystallized from EtOAc for NMR and CHN analysis.

- 10 $^1\text{H-NMR}$ (CDCl_3) δ 2.28 (s, 3H, 2- CH_3), 3.80 (s, 3H, $\text{CH}_3\text{N-}$), 3.89 (s, 3H, $\text{CH}_3\text{O-}$), 4.86 (s, 2H, CH_2Cl) and 5.62 (s, 1H, 6- H). Anal. C; 55.71, H; 5.41, N; 5.70, Cl; 14.00% Calc. $\text{C}_{12}\text{H}_{12}\text{NO}_3\text{Cl} \cdot 0.33\text{H}_2\text{O}$ C; 55.49, H; 4.88, N; 5.39, Cl; 13.68%

GENERAL METHOD FOR THE SYNTHESIS OF TARGET COMPOUNDS OF TYPE A.

- 15 5-Methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione (52, 500mg, 1.97mmol) was dissolved in CH_2Cl_2 (50mL) together with pyridine (5mL) and the appropriate carbonyl chloride (5mmol) added. The solution was heated under reflux for the reaction time given below, cooled and EtOAc (150mL) added followed by HCl (aq., 0.1M, 150mL). The organic layer was separated and washed again with HCl (aq., 0.1M, 100mL),
20 sat. NaCl (aq., 100mL), dried and evaporated *in vacuo*. The residue was purified on silica, eluting with the solvent specified below and recrystallized to give the target compound.

B : GENERAL METHOD FOR THE SYNTHESIS OF TARGET COMPOUNDS OF TYPE B AND TYPE C

- 25 3-Chloromethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (67, 100mg, 0.39mmol) was dissolved in EtOAc (20mL) and the appropriate alcohol, phenol or thiol (1mmol) added dropwise or in portions with stirring. Stirring was continued at room temperature for the

reaction times given below and then water (20mL) added. The organic layer was separated and washed with sat. NaHCO₃ (aq., 20mL) and sat. NaCl (aq., 20mL), dried and evaporated to dryness. The residue was purified on silica, eluting with the solvent specified below, and recrystallized to give the target compound.

5 **Example 33: Preparation of 1,2-Dimethyl-5-methoxy-3-(2-nitrobenzoyloxy)-ethylindole-4,7-dione (68)**

General method A was employed (reaction time 0.75h), and the product eluted on silica with EtOAc (R_f=0.65), and recrystallized from EtOAc to give an orange solid (68%) :
mp 199-200°C; ¹H-NMR (CDCl₃) δ 2.34 (s, 3H, 2-CH₃), 3.80 (s, 3H, CH₃N), 3.91 (s,
10 3H, CH₃O), 5.53 (s, 2H, CH₂OCOAr), 5.62 (s, 1H, 6-H), 7.50-8.07 (m, 4H, 4 x Ar-H).
Anal.

Example 34: Preparation of 1,2-Dimethyl-3-(2-fluorobenzoyloxy)methyl-5-methoxyindole-4,7-dione (69).

General method A was employed (reaction time 1.5h), and the product eluted on silica
15 with EtOAc (R_f=0.6), and recrystallized from EtOAc to give a yellow solid (83%):
mp 166-168°C; ¹H-NMR (CDCl₃) δ 2.35 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N), 3.90 (s,
3H, CH₃O), 5.51 (s, 2H, CH₂OCOAr), 5.63 (s, 1H, 6-H), 6.98-7.52 (m, 3H, 3 x Ar-H)
and 7.83-8.00 (dd, 1H, J=9Hz and J=1.5Hz, Ar3-H). Anal. C; 62.83, H; 4.59, N; 3.76%
Calc. (C₁₉H₁₆NO₅F.0.33H₂O) C; 62.81, H; 4.59, N; 3.86%

20 **Example 35: Preparation of 1,2-Dimethyl-3-(4-fluorobenzoyloxy)methyl-5-methoxyindole-4,7-dione (70).**

General method A was employed (reaction time 1.25h), and the product eluted on silica
with EtOAc (R_f=0.65), and recrystallized from EtOAc to give a yellow solid (88%) :
mp 209-210°C; ¹H-NMR (CDCl₃) δ 2.35 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N), 3.91 (s,
25 3H, CH₃O), 5.49 (s, 2H, CH₂OCOAr), 5.63 (s, 1H, 6-H), 6.95-7.27 (dd, 2H, J=9Hz and
J=1Hz, Ar2,6-H) and 7.94-8.10 (dd, 2H, J=9Hz and J=1.5Hz, Ar3,5-H).
Anal. C; 63.34, H; 4.44, N; 3.88% Calc. (C₁₉H₁₆NO₅F) C; 63.86, H; 4.51, N; 3.92%.

Example 36: Preparation of 3-Benzoyloxymethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (71)

General method A was employed (reaction time 1.5h), and the product eluted on silica with EtOAc ($R_f=0.75$), and recrystallized from EtOAc/Me₂CO to give a yellow solid
5 (69%) : mp 168-170°C; ¹H-NMR (CDCl₃) δ 2.31 (s, 3H, 2-CH₃), 3.76 (s, 3H, CH₃N), 3.86 (s, 3H, CH₃O), 5.46 (s, 2H, CH₂OCOAr), 5.58 (s, 1H, 6-H), 7.34-7.41 (m, 3H, 3 x ArH) and 7.93-8.01 (m, 2H, 2 x Ar-H).

Example 37: Preparation of 3-(2-Acetoxybenzoyloxy)-methyl-1,2-dimethyl-5-methoxyindole-4,7-dione (72).

10 General method A was employed (reaction time 1.5h), and the product eluted on silica with EtOAc ($R_f=0.5$), and recrystallized from EtOAc to give a yellow solid (90%): mp 159-161°C; ¹H-NMR (CDCl₃) δ 2.27 (s, 3H, CH₃CO₂Ar), 2.31 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N), 3.90 (s, 3H, CH₃O), 5.47 (s, 2H, CH₂OCOAr), 5.63 (s, 1H, 6-H), 7.01-7.53 (m, 3H, 3 x ArH) and 7.94-8.04 (dd, 1H, $J=9$ Hz and $J=1$ Hz, Ar-H).
15 Anal. C; 63.81, H; 4.81, N; 3.71% Calc. (C₂₁H₁₉NO₇) C; 63.47, H; 4.82, N; 3.52%

Example 38: Preparation of 3-Acetoxymethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (73)

General method A was employed (reaction time 0.5h), and the product eluted on silica with EtOAc ($R_f=0.6$), and recrystallized from EtOAc to give a yellow solid (90%):
20 mp 185-186°C; ¹H-NMR (CDCl₃) δ 2.04 (s, 3H, CH₃CO), 2.28 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N), 3.90 (s, 3H, CH₃O), 5.24 (s, 2H, CH₂OCOCH₃) and 5.62 (s, 1H, 6-H).

Example 39: reparation of 3-(Cyclohexylcarbonyloxy)-methyl-1,2-dimethyl-5-methoxyindole-4,7-dione (74).

25 General method A was employed (reaction time 0.25h), and the product eluted on silica with EtOAc ($R_f=0.75$), and recrystallized from EtOAc to give a yellow solid (65%) : mp 164-165°C; ¹H-NMR δ 1.2-1.83 (m, 11H, 11 x cyclohexyl-H), 2.27 (s, 3H, 2-CH₃), 3.80 (s, 3H, CH₃N), 3.89 (s, 3H, CH₃O), 5.22 (s, 2H, CH₂OCOR) and 5.62 (s, 1H, 6-H).

Example 40: Preparation of 1,2-Dimethyl-3-(4-nitrophenoxy)-methyl-5-methoxyindole-4,7-dione (75).

General method B was employed (reaction time 0.5h), and the product eluted on silica with EtOAc/hexane (1:1, Rf=0.7), and recrystallized from EtOAc to give an orange
 5 solid (54%) : mp 215-216°C (dec.); ¹H-NMR d 2.33 (s, 3H, 2-CH₃), 3.81 (s, 3H, CH₃N), 3.89 (s, 3H, CH₃O), 4.99 (s, 2H, CH₂OAr), 5.62 (s, 1H, 6-H) and 6.79-6.93 (m, 4H, 4 x Ar-H).

Example 41: Preparation of 1,2-Dimethyl-3-(4-fluorophenoxy)-methyl-5-methoxyindole-4,7-dione (76).

10 General method B was employed, and the product eluted on silica with EtOAc/hexane (1:1, Rf=), and recrystallized from EtOAc to give an solid (40%).

Example 42: Preparation of 1,2-Dimethyl-3-(2-fluorophenoxy)-methyl-5-methoxyindole-4,7-dione (77).

General method B was employed, and the product eluted on silica with EtOAc/hexane
 15 (1:1, Rf=), and recrystallized from EtOAc to give an solid (55%).

Example 43: Preparation of 3-(2-Carbomethoxythiophenyl)-methyl-1,2-dimethyl-5-methoxyindole-4,7-dione (78).

General method B was employed (reaction time 2h), and the product eluted on silica with EtOAc/hexane (1:1, Rf=0.25), and recrystallized from EtOAc to give an orange
 20 solid (66%) : mp 214-216°C ; ¹H-NMR (CDCl₃) d 2.21 (s, 3H, 2-CH₃), 3.77 (s, 3H, CH₃N), 3.86 (s, 6H, CH₃O and CO₂CH₃), 4.39 (s, 2H, CH₂SAr), 5.59 (s, 1H, 6-H), 7.28-7.49 (m, 3H, 3 x ArH) and 7.95 (d, 1H, J=9Hz, Ar-H).

Example 44: Preparation of 3-Benzylloxymethyl-1,2-dimethyl-5-methoxyindole-4,7-dione (79).

25 General method B was employed (reaction time 1h) but with the addition of K₂CO₃ (138mg, 1mmol) to the reaction mixture, and the product eluted on silica with

EtOAc/hexane (1:1, Rf=0.6), and recrystallized from EtOAc to give an orange solid (53%): mp 132-133°C (dec.); ¹H-NMR (CDCl₃) δ 2.25 (s, 3H, 2-CH₃), 3.80 (s, 3H, CH₃N), 3.86 (s, 3H, CH₃O), 4.59 (s, 2H, 3-CH₂OCH₂Ar), 4.74 (s, 2H, 3-CH₂OCH₂Ar), 5.60 (s, 1H, 6-H) and 7.28-7.36 (m, 5H, 5 x ArH). Anal. C; 67.81, H; 5.95, N; 4.49% Calc. (C₁₉H₁₉NO₄·0.5H₂O) C; 68.26, H; 5.99, N; 4.19%

Example 45: Preparation of 3-Benzoyloxymethyl-2-cyclopropyl-5-methoxy-1-methylindole-4,7-dione (80): 2-cyclopropane derivative.

General method A was employed (reaction time 1.5h) using compound 20, and the product eluted on silica with EtOAc/hexane (1:1, Rf=0.35), and recrystallized from EtOAc/Me₂CO to give a yellow solid (68%): mp 221-223°C; ¹H-NMR (CDCl₃) δ 0.65-0.82 (m, 2H, cyclopropyl-CH₂), 1.11-1.23 (m, 2H, cyclopropyl-CH₂), 1.61-1.71 (m, 1H, cyclopropyl-H), 3.79 (s, 3H, CH₃N), 4.03 (s, 3H, CH₃O), 5.56 (s, 2H, CH₂OCOAr), 5.64 (s, 1H, 6-H), 7.31-7.49 (m, 3H, 3 x ArH) and 7.96-8.08 (m, 2H, 2 x Ar-H). Anal. C; 69.04, H; 5.30, N; 3.82% Calc. (C₂₁H₁₉NO₅) C; 69.03, H; 5.24, N; 3.83%.

Example 46: 5-(2-Methylaziridin-1-yl)-3-(2-nitrobenzoyloxymethyl)-1,2-dimethylindole-4,7-dione (81).

Compound 3 (0.1g, mmol) was dissolved and stirred in freshly distilled 2-methylaziridine (3mL, ca.50mmol) for 2.5h. The solution was evaporated *in vacuo* and the residue redissolved in EtOAc, evaporated until a red precipitate appeared and the solid collected. The red solid was recrystallized from EtOAc to give 81 (85mg, %): mp 168-170°C; ¹H-NMR (CDCl₃) δ 1.42 (d, 3H, J=4.5Hz, azir-CH₃), 2.01-2.15 (m, 3H, azir-CHCH₂), 2.33 (s, 3H, 2-CH₃), 3.90 (s, 3H, CH₃N-), 5.54 (s, 2H, CH₂OCOAr), 5.73 (s, 1H, 6-H), 7.53-8.01 (m, 4H, 4 x Ar-H).

Example 47: Preparation of 5-(2-Methylaziridin-1-yl)-3-benzoyloxymethyl-1,2-dimethylindole-4,7-dione (82).

This compound was prepared from 6 by the same method used for the synthesis of 82.

The red solid was recrystallized from EtOAc to give 17 (70 %): mp 133-134°C;

- 5 $^1\text{H-NMR}$ (CDCl_3) δ 1.42 (d, 3H, $J=4.5\text{Hz}$, azir- CH_3), 2.01-2.15 (m, 3H, azir- CHCH_2), 2.33 (s, 3H, 2- CH_3), 3.90 (s, 3H, $\text{CH}_3\text{N-}$), 5.54 (s, 2H, CH_2OCOAr), 5.73 (s, 1H, 6- H), 7.34-7.41 (m, 3H, 3 x Ar H) and 7.93-8.01 (m, 2H, 2 x Ar- H). Anal. C; 67.96, H; 5.67, N; 7.23%, Calc. ($\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C; 67.56, H; 5.63, N; 7.51%.

10 **Example 48: Preparation of 1,2-Dimethyl-3-(4-methylpiperazin-1-yl)methyl-5-methoxyindole-4,7-dione (83).**

General method B was employed (reaction time 1h) but using N-methylpiperazine (0.65mL, 0.58mmol) in place of the alcohol or thiol, and the product eluted on silica with MeOH ($R_f=0.15$) to give 83 as an orange solid (38%): mp 159-162°C (dec.);

- 15 $^1\text{H-NMR}$ (CDCl_3) δ 2.24 (s, 6H, 2- CH_3 and N- CH_3), 2.31-2.62 (m, 8H, 4 x piperazine- CH_2), 3.73 (s, 2H, 3- CH_2N), 3.79 (s, 3H, CH_3N), 3.88 (s, 3H, CH_3O) and 5.69 (s, 1H, 6- H).

Example 49: Preparation of 5-(Aziridin-1-yl)-1,2-dimethyl-3-(4-methylpiperazin-1-yl)methylindole-4,7-dione (84).

Compound 83 (50mg, 0.158mmol) was stirred for 0.3h with 1H-aziridine (CAUTION!)

- 20 at room temperature. Excess aziridine was removed *in vacuo* and the residue twice redissolved in EtOAc (5mL) and evaporated to dryness. The residue was recrystallised from EtOAc to give 84 as a dark red solid (mg, 62%): mp 143-146°C (dec.); $^1\text{H-NMR}$ (CDCl_3) δ 2.17 (s, 4H, aziridine), 2.25 (s, 6H, 2- CH_3 and N- CH_3), 2.31-2.62 (m, 8H, 4 x piperazine- CH_2), 3.74 (s, 2H, 3- CH_2N), 3.87 (s, 3H, CH_3N), and 5.73 (s, 1H, 6- H).

The amount of compound to be administered will of course vary with individual and cancer type. However, suitable doses will be typically within the range 1-200mg/kg bodyweight, preferably 10-150mg/kg and more preferably 30-100mg/kg.

The form of administration will also vary, with formulations suitable for oral, 5 rectal, topical, parenteral (including subcutaneous, intramuscular and intravenous injection) being useable. Formulations may range from pure drug and simple aqueous solutions thereof, eg. in water or saline, to those including solid and/or more complex components such as liquid or solid carriers, diluents and active agents. Particular forms available include *inter alia* capsules, cachets, tablets or lozenges, boluses, electuaries or 10 pastes containing solid or liquid compound of the invention, or suspensions, syrups, emulsions etc in liquid form. Suitable formulations will occur to those skilled in the art of pharmacy and may be exemplified by those used for the current lead EO9 and the antimicrobial agents described previously.

Simple saline injections may be used for the purpose of demonstrating efficacy 15 of the compounds.

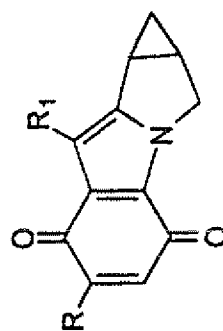
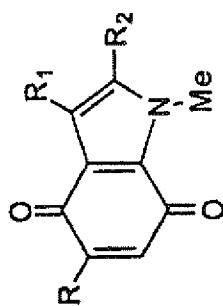
Biological Evaluation of Compounds 18 to 84 *In Vitro*














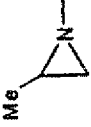


Example 50: MTT assay




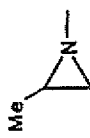




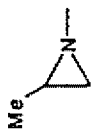




Selective toxicity to hypoxic V79-379A cells was determined for all compounds using the MTT assay as has been described previously (see references 7 to 9). These 20 results are presented in the Table 1 below, where C_{50} (air) values, the concentration required to kill 50% of the aerobic cells under the conditions of the assay, are divided by C_{50} (N_2) values, concentrations required to kill hypoxic cells, to give hypoxic cytotoxicity ratios (HCR), which enable quantitative comparisons of bioreductive activities of drugs.




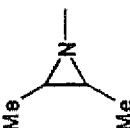


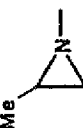

TABLE I Compounds 1 to 66

		A				B		HCR*
Type	R	R ₁	R ₂	C ₅₀ (Air)	C ₅₀ (N ₂)	μM	μM	
1	MMC	-	-	0.8 ^a	0.4 ^a	0.4 ^a	2.0 ^a	
2	A	CH ₂ OCONH ₂	-	0.003 ^b	0.003 ^b	0.003 ^b	1.0 ^b	
3	EO9	-	-	0.19±0.027	0.0038±0.00057	0.0038±0.00057	50.3	



4	A	MeO	CH ₂ OH	-	108.4±5.9	60.6±7.4	1.8
5	A		CH ₂ OH	-	0.965±0.11	0.073±0.011	13.2
18	B	MeO	CO ₂ Me		103.5±5.2	59.8±11.4	1.7
20	B	MeO	CH ₂ OH		540±52	820±83	0.65
23	B	MeO	CH ₂ OCO ₂ Ph		250±24	60±9.8	4.2
24	B	MeO	CH ₂ OCONH ₂		29±5.6	0.44±0.023	65.9
19	B		CO ₂ Me		1.72±0.18	0.93±0.067	1.8
21	B		CH ₂ OH		0.603±0.099	0.0058±0.0013	103.5
25	B		CH ₂ OCONH ₂		3.33±0.75	0.074±0.015	45.0
22	B		CH ₂ OH		130±9.8	5.6±0.6	23.4
26	B		CH ₂ OCONH ₂		12.5±2.9	0.459±0.096	27.23
35	B	MeO	CO ₂ Me		150±15	150±15	1.0

36	B	MeO	CH ₂ OH		800±80	200±20	4.0
37	B		CH ₂ OH		25.7±3.0	0.79±0.14	32.5
38	B		CH ₂ OH		127.8±13.5	13.9±1.5	9.2
40	B	MeO	CH ₂ OC(=O)NH ₂		20.9±1.67	0.117±0.018	178.6
41	B		CH ₂ OC(=O)NH ₂		47.5±14.6	5.46±0.43	8.7
42	B		CH ₂ OC(=O)NH ₂		20.6±2.5	0.87±0.215	23.7
43	B	MeO	Me		1.236±0.189	0.657±0.063	1.88
44	B		Me		0.333±0.014	0.188±0.011	1.77
45	B	MeO	CH ₂ OH	Cyclohexyl	122.4±17.5	20.5±5.3	6.0

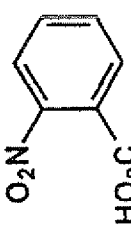
46	B		CH ₂ OH	Cyclohexyl	34.5±6.0	0.93±0.078	37.0
52	B	MeO	CH ₂ OH	Me	1077±44	284.8±37.6	3.78
53	B		CH ₂ OH	Me	0.149±0.011	0.0116±0.0008	12.8
54	B		CH ₂ OH	Me	94±7.6	0.5±0.07	188
55	B		CH ₂ OH	Me	202±11	14.2±1.7	14.2
56	B		CH ₂ OH	Me	1260±126	500±120	2.5
62	B	MeO	CH ₂ OH	H	220±20	240±23	0.92
63	B		CH ₂ OH	H	0.153±0.013	0.0086±0.0009	15.4
64	B		CH ₂ OH	H	4.42±1.17	0.0179±0.02	24.7
65	B	MeO	CH ₂ OCONH ₂	H	3.1±0.24	0.037±0.007	83.8
66	B		CH ₂ OCONH ₂	H	0.00019±0.000032	0.00013±0.000025	1.46

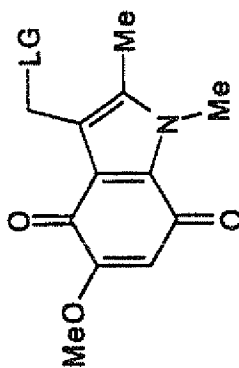
*HCR = hypoxic cytotoxicity ratio ($C_{50}(\text{Air})/C_{50}(\text{N}_2)$).

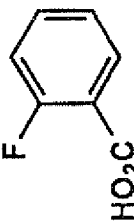
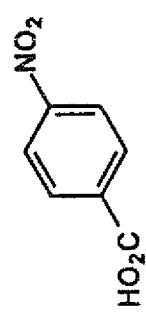
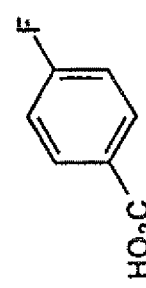
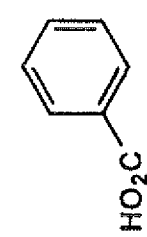
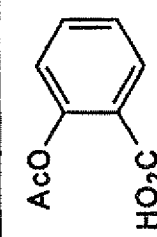
^a Stratford, I.J. *et al.* (1990), in *Selective Activation of Drugs by Redox Processes*; Adams, G.E. *et al.*, Eds., Plenum, N.Y.

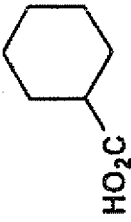
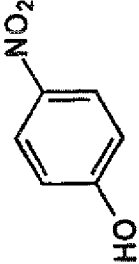
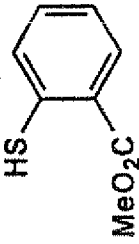
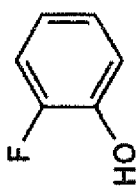
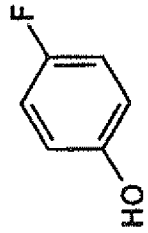
^b Moody, C.J. *et al.* (1994), *Anti-Cancer Drugs*, **5**, 367-372.

TABLE 2: LEAVING GROUP ACID DISSOCIATION CONSTANTS AND HCR (MTT) OF DRUGS WITH VARYING INDOLQUINONE 3-METHYLENE LEAVING GROUPS

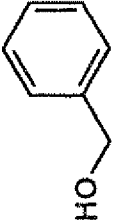
Compound	LG(H)	pK _a (H ⁺) ^{1,2}		C ₅₀ N ₂	HCR
RB94564J	HCl	-2.2	-	-	-
67					
RB96709J		2.21	158.9	11.4	14.0
68			±13.0	±1.54	



RB96716N 69		3.2	163.8 ±24.7	2.06 ±0.25	79.5
<hr/>					
		3.42			
<hr/>					
RB96715N 70		3.98	27.13 ±3.12	0.29 ±0.03	93.5
<hr/>					
RB96708N 71		4.14	16.14 ±5.14	0.197 ±0.045	82.0
<hr/>					
RB96714N 72		4.5	29.4 ±3.8	1.38 ±0.14	21.3
<hr/>					
RB96717N 73	AcOH	4.75	27.3 1.54	2.45 ±0.18	11.1

RB96727N		4.9 ³			
74					
RB96713J		7.15	72.9	1.15	63.4
75			±19.3	±0.136	
RB96725N		7.8 ⁴	319.6	369.1	0.86
78			±91.8	±77.1	
RB96734N		8.81	472.0	282.4	1.7
77			±37.5	±107.3	
RB96728N		9.81			
76					

RB94547J

50	H ₂ O	15.74	1077.0 ±44.0	284.8 ±37.6	3.78
<hr/>					
RB96712J					
79		15.9 ⁵	225.1 ±14.6	412.0 ±31.8	0.55

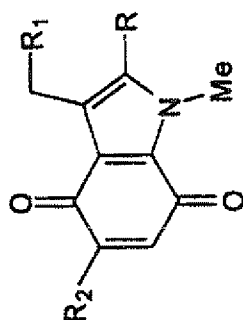
1. Streitwieser, A.; Heathcock, C. H. In *Introduction to Organic Chemistry*, Macmillan Pub. Co., Inc., New York, 1981.

2. Weast, R.C.; Astle, M. J. In *Handbook of Chemistry and Physics* (62nd edn.) 1981-82.

3, 4, 5 Values for C₂H₅CO₂H, C₆H₅SH and C₂H₅OH from ref. 1.

Other compounds prepared include those where the leaving group is 2,4,6 trichloro phenol and 4-dimethylamino benzoic acid having pK_a (H⁺) of 6.0 and 4.92 respectively.

TABLE 3: DATA AVAILABLE ON COMPOUNDS 80-84



Compound	R	R ₁	R ₂	C ₅₀ Air	C ₅₀ N ₂	HCR
80	cyclopropyl	benzoyloxy	MeO	197±68.9	1.01±0.07	196
81	methyl	2-nitrobenzoyloxy	2-methylaziridin-1-yl	36.2±2.7	1.05±0.15	34.5
82	methyl	benzoyloxy	2-methylaziridin-1-yl	1.22±0.16	0.062±0.0071	19.7
84	methyl	4-methylpiperazin-1-yl	aziridin-1-yl	11.01±1.12	0.025±0.0026	440

Example 52: Effect of compounds on RIF-1 and KHT tumour cells when administered with single dose radiation regimen.

Compounds 21 and 54 (RB 94573 and RB 94577) were applied to RIF-1 and KHT tumour cells in separate experiments with presence and absence of single doses of radiation (15GY and 10 GY respectively for 21 and 54). Maximum tolerated doses and tumour response experiments were carried out as described in reference 9. Tumour bearing mice were given doses of radiation chosen to kill most of the oxic cells in the tumours with the measured response reflecting the survival of residual hypoxic clonogenic cells (see reference 10). Indoquinones were given immediately after X-rays such that any therapeutic response greater than that achieved by radiation alone is a reflection of residual hypoxic cell killing. These conditions were achieved by giving 10GY to KHT tumours and 25 GY to RIF-1 tumours. Each experiment also included mice exposed to radiation without drug and with drug alone.

Results are shown in Figures 6 to 9. It can be seen that while the results are additive in the case of the KHT cells, providing destruction of tumour cells down to a surviving fraction of as low as less than 10^{-4} , the RIF-1 cells show a synergistic effect with surviving fraction being less than 10^{-6} .

Example 53: Effect of compound 54 (RB 94577) with fractionated dose radiation regimen.

Four daily doses of 2.5 Gy of X-rays were administered to RIF-1 cells followed immediately by 20mg/Kg of compound 52 after each fraction. Results are given in the Table 4 below.

TABLE 4 **RB94577 (54) + X-rays in fractionated treatments**

4 daily doses of 2.5 Gy X-rays followed immediately by 20 mg/kg RB94577 after each fraction. Tumours were excised 24 hours after last dose of drug to assay cell survival.

<u>Treatment</u>	<u>Relative surviving fraction</u>
Control	1.0
4 x 20 mg/kg RB94577	0.13
4 x 2.5 Gy	3.3×10^{-2}
RB94577 + X-rays	1.6×10^{-3}

TABLE 5

Effect of fractionated treatments with RB94577 (54) and radiation on RIF-1 tumours.

<u>Treatment</u>	<u>Relative Surviving Fraction</u>
Control-No compound or radiation	4x 20mg/kg/day
4x 30 mg/kg/day	4x 40 mg/kg/day
1.00	0.13
1.3×10^{-2}	0.9×10^{-3}

TABLE 6

Interaction between RB94577 (54) and cisPlatin in RIF tumour-no-radiation.

<u>Treatment</u>	<u>Relative Surviving Fraction</u>
50 mg/kg Compound 54 alone	0.1
3 mg/kg cisPlatin alone	5×10^{-2}
Compound 54+cisPlatin (5min*)	4.6×10^{-4}
Compound 54+cisPlatin (60min*)	3.3×10^{-4}
cisPlatin+Compound 54 (60min*)	7.9×10^{-4}

*The time between administration of the first mentioned agent and the second is indicated in minutes. Effect of the compound of the invention and cisPlatin is greater than additive in this assay.

TABLE 7:**Compound 53 as compared to EO9.**

Cell line	Origin	DTDActivity	EO9IC ₅₀	Cmpd53IC ₅₀
A549	NSCLC	4980	7	6.2
322	NSCLC	4510	15	79.7
HT29	Colon	2750	157	82.3
522	NSCLC	550	32	17.5
841	SCLC	440	51	16.4
417	SCLC	116	210	155
MDA468	Breast	63	1720	3648
T47D	Breast	39	5110	1826
MDA231	Breast	8	285	1041
249	SCLC	2.5	3710	1804

DTD= DT diaphorase activity in nmol cytochrome c reduced per min per mg protein

IC₅₀ (nM) concentration required to kill 50% of cells following four days incubation of cells with drug in air.

Table 8 IC₅₀ (µm) for various quinones on various cell lines.

Compound	MDA231			MDA468			A549			HT29			SCCVII			V79		
	Air	N ₂	HCR	Air	N ₂	HCR	Air	N ₂	HCR	Air	N ₂	HCR	Air	N ₂	HCR	Air	N ₂	HCR
RB94573	2	0.5	4	-	-	-	0.02	0.02	1.0	-	-	-	-	-	-	0.6	0.006	100
RB94577	40	10	4	-	-	-	-	-	-	-	-	-	0.1	0.01	10	94	0.5	190
RB94582*	1.5	0.5	3	0.8	0.02	4	1.0	1.0	1.0	-	-	-	5	0.3	17	30	0.4	75
RB95662**	4.1	0.3	14	14	-	-	0.7	0.13	5.0	3.4	0.8	4.0	1.9	0.03	70	11	0.025	440

* Compound 24

**Compound 84

TABLE 9**Comparative tumour toxicities (relative surviving fraction) of compounds 21 and 54.**

<u>Tumour</u>	<u>RB95477-Compound 54</u>	<u>RB94573-Compound 21</u>
KHT	1.2×10^{-1}	3.6×10^{-2}
RIF	1.5×10^{-2}	8.6×10^{-2}
SCCVII	2.3×10^{-2}	5.6×10^{-3}

Both drugs were given at 90 mg/kg ip and tumours were excised at 24 hours.

TABLE 10**Effects of Compound 54 as compared to EO9 of US 5097257.**

<u>Tumour</u>	<u>Diaphorase*</u>	<u>Control</u>	<u>4x vol-Cd 54</u>	<u>4xvol-EO9</u>
H249	44	11.4	15.9	22.0
HT29	1544	11.4	19.5	17.4
H647	1960	12.2	13.5	21.1

*Activity in nmol cytochrome c reduced per min per mg protein

Compound 54 was given at 30 mg/kg i.p. while EO9 was given at 5 mg/kg. The maximum tolerated dose of Compound 54 is about 100 mg/kg while the max tolerated dose of EO9 is 5 mg/kg. H249 is human small cell cancer type; HT29 is human colon cancer type and H647 is human non-small cell lung cancer type.

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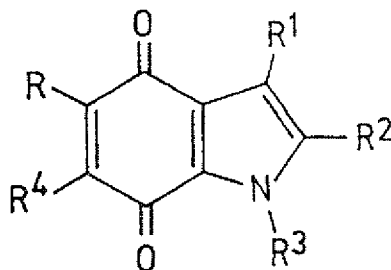
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CLAIMS

1. Use of a compound of general formula I



or salt thereof

wherein

R and R⁴ are independently selected from hydrogen, halogen and C₁₋₆ alkyl or

5 haloalkyl, C₂₋₆ alkenyl or haloalkenyl, C₁₋₆ alkoxy, phenoxy, C₁₋₆ alkylthio, phenylthio, primary and secondary amino or hydroxy groups and

R³ is hydrogen, hydroxy, a C₁₋₆ alkyl or haloalkyl, C₂₋₆ alkenyl or haloalkenyl or C₁₋₆ alkoxy or haloalkoxy group for the manufacture of a medicament for the treatment of neoplasms

10 characterised in that

R¹ is selected from a C₁₋₆ alkyl or haloalkyl group, -CO₂R⁵ where R⁵ is hydrogen or a C₁₋₆ alkyl or haloalkyl group, or a group -CH₂-X where X is selected from groups of

15 formula -S-R⁶, -O-R⁶ and -N $\begin{matrix} \nearrow R^7 \\ \searrow R^8 \end{matrix}$ where R⁶ is a hydrogen or a leaving group the acid HR⁶ of which has a pK_a of 10 or less and R⁷ and R⁸ are the same or different and are selected from C₁₋₆ alkyl or haloalkyl or together with the interjacent nitrogen form a heterocyclic ring of 5 to 7 atoms optionally substituted by C₁₋₄ alkyl or haloalkyl and

20 R² is selected from hydrogen, C₁₋₄ alkyl and haloalkyl or groups -(CH₂)_nCHR⁹R¹⁰ of more than four carbon atoms where n is an integer of 0 to 2 and R⁹ and R¹⁰ are independently selected from a C₁₋₄ alkyl or haloalkyl group, or R⁹ and R¹⁰ together with the interjacent carbon atom form a C₃₋₇ cycloalkyl or cycloalkenyl ring optionally substituted with one or more C₁₋₄ alkyl or haloalkyl, or C₂₋₄ alkenyl or haloalkenyl

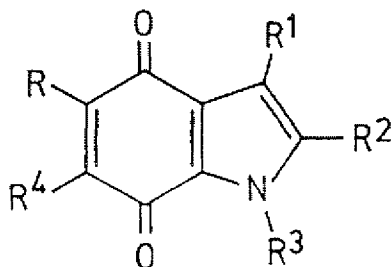
groups.

2. Use of a compound as claimed in Claim 1 characterised in that R^1 is a group
 $-CH_2X$ where X is $-N \begin{smallmatrix} R^7 \\ R^8 \end{smallmatrix}$ wherein R^7 and R^8 , together with the interjacent
 5 nitrogen form a 5 to 7 membered heterocyclic ring containing nitrogen and carbon with
 optional oxygen or sulphur members.
3. Use of a compound as claimed in Claim 2 characterised in that the heterocyclic
 ring is a piperazinyl ring optionally substituted by one or more C_{1-4} alkyl or haloalkyl
 10 groups.
4. Use of a compound as claimed in Claim 3 wherein X is a 4-methylpiperazin-1-yl
 group.
5. Use of a compound as claimed in Claim 1 characterised in that R is selected
 from C_{1-4} alkoxy, C_{1-4} haloalkoxy, C_{2-4} alkenoxy, C_{2-4} haloalkenoxo and
 15 aziridin-1-yl optionally substituted with C_{1-4} alkyl, or C_{1-4} haloalkyl or
 C_{2-4} alkenyl or C_{2-4} haloalkenyl;
 R^1 is selected from C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} hydroxyalkyl,
 C_{1-4} alkoxycarbonyl, C_{1-4} haloalkoxycarbonyl and C_{1-4} alkyl substituted with an
 alkoxy, aryloxy, carbonyloxy, aryloxy, carbonyloxy or carbamoyloxy group;
 20 R^2 is selected from $-(CH_2)_nCHR^9R^{10}$ where n is an integer from 0 to 2, and R^9 and
 R^{10} are independently selected from C_{1-4} alkyl and C_{1-4} haloalkyl or together
 with the interjacent carbon atom form a C_{3-6} cycloalkyl or cycloalkenyl group
 optionally substituted with C_{1-4} alkyl, C_{1-4} haloalkyl, C_{2-4} alkenyl or
 C_{2-4} haloalkenyl;
 25 R^3 is selected from C_{1-4} alkyl or haloalkyl and C_{2-4} alkenyl or haloalkenyl;
 and R^4 is selected from hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{2-4} alkenyl and
 C_{2-4} haloalkenyl.

6. Use of a compound as claimed in any one of Claims 1 to 5 characterised in that R is C₁₋₄ alkoxy or is aziridin-1-yl optionally substituted with C₁₋₄ alkyl or C₁₋₄ haloalkyl.
7. Use of a compound as claimed in any one of the preceding claims characterised in that R¹ is a group -CH₂-O-R⁶ or -CH₂-S-R⁶ and R⁶ is hydrogen or a group -C(O)-R¹¹ or -R¹¹ where R¹¹ is an optionally substituted phenyl, benzyl or amino group.
8. Use of a compound as claimed in Claim 5 characterised in that R¹ is hydroxymethyl or a carbamate or aromatic acyl ester thereof.
9. Use of a compound as claimed in Claim 1 characterised in that R² is a C₁₋₄ n-, iso- or cyclo-alkyl group.
10. Use of a compound as claimed in Claim 5 characterised in that R² is a methyl or cyclopropyl group.
11. Use of a compound as claimed in Claim 1 characterised in that R³ is C₁₋₄ alkyl.
12. Use of a compound as claimed in Claim 1 characterised in that R⁴ is hydrogen.
13. Use of a compound as claimed in Claim 1 where R is aziridin-1-yl optionally substituted with C₁₋₄ alkyl, R¹ is hydroxymethyl, R² is cyclopropyl or isopropyl or C₁₋₄ n-alkyl, R³ is methyl and R⁴ is hydrogen.
14. Use of a compound as claimed in Claim 1 characterised in that the compound has a ratio of C₅₀(Air)μM/C₅₀(N₂)μM of 40 or more.
15. Use of a compound as claimed in Claim 14 characterised in that it has a ratio of

$C_{50}(\text{Air})\mu\text{M}/C_{50}(\text{N}_2)\mu\text{M}$ of 100 or more.

16. Use of a compound specifically as exemplified and described herein as any one of compounds 18 to 84 .
17. Use of a compound as claimed in Claim 16 and as defined herein as
 5 compound 21, 22, 24, 25, 26, 37, 40, 42, 46, 53, 54, 55, 63, 64, 65, 68, 69, 70, 71, 72, 73, 75, 80, 81, 82, 83 or 84 of the Examples.
18. Use as claimed in any one of the preceding claims characterised in that the use is for the treatment of tumour cells.
19. Use as claimed in any one of the preceding claims characterised in that the use is
 10 for the treatment of cancer cells.
20. Use as claimed in any one of the preceding claims characterised in that the use is provided together with radiation treatment as a therapeutic.
21. Use as claimed in any one of the preceding claims characterised in that the use
 15 provides selective cytotoxicity to anoxic tumour cells.
22. A compound of general formula Ia



or a salt thereof characterised in that

R is C₁₋₄ alkoxy or aziridin-1-yl optionally substituted with one or more C₁₋₄ alkyl or

haloalkyl groups,

R^3 is hydrogen, hydroxy, C_{1-6} alkyl or haloalkyl, C_{2-6} alkenyl or haloalkenyl or C_{1-6} alkoxy or haloalkoxy

R^4 is hydrogen or a C_{1-4} alkyl or haloalkyl group,

5 R^5 is hydrogen, C_{1-6} alkyl or haloalkyl, C_{2-6} alkenyl or haloalkenyl or C_{1-6} alkoxy,

R^1 is $-CO_2R^5$ where R^5 is hydrogen or a C_{1-6} alkyl or haloalkyl group, or is a group

$-CH_2-X$ where X is selected from groups of formula $-SR^6$, $-O-R^6$ and $-N \begin{matrix} R^7 \\ R^8 \end{matrix}$ where R^6

10 is hydrogen or a leaving group the acid HR^6 of which has a pKa of 10 or less and R^7 and R^8 are the same or different and are selected from C_{1-6} alkyl or together with the interjacent nitrogen form a heterocyclic ring of 5 to 7 atoms optionally substituted by a C_{1-4} alkyl group and

R^2 is hydrogen or a C_{1-4} alkyl group or a group $-(CH_2)_nCHR^9R^{10}$ of more than four

15 carbons where n is an integer of 0 to 2 and R^9 and R^{10} are independently selected from C_{1-4} alkyl and haloalkyl or R^9 and R^{10} together with the interjacent carbon atom form a C_{3-7} cycloalkyl or cycloalkenyl ring optionally substituted with one or more C_{1-4} alkyl or haloalkyl or C_{2-4} alkenyl or haloalkenyl groups,

with the provisos that

20 (i) when R is methoxy, R^1 is hydroxymethyl or a carbamate or C_{1-6} aliphatic acyl ester thereof, R^4 is methyl or ethyl and R^3 is methyl, optionally 2-substituted ethyl, or propyl or butyl then R^2 is not hydrogen, methyl, fluoromethyl, chloromethyl or ethyl,

(ii) when R is methoxy, R^4 is hydrogen, R^1 is hydroxymethyl or the propylcarbamate thereof and R^3 is ethyl then R^2 is not methyl,

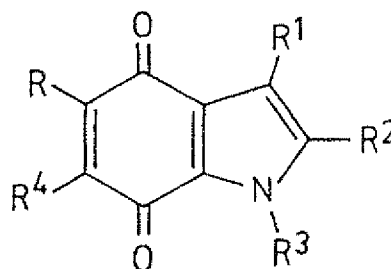
25 (iii) when R is methoxy, R^2 is methyl, R^3 is ethyl and R^4 is methyl, R^1 is not a hydroxymethyl cyclohexylcarboxylate, benzoate, furamyl-2-carboxylate, 3-(2-dimethyl-aminoethyl)-piperazine-1-carboxylate, morpholino carbamate, 4-(3-hydroxypropyl)-piperazine carbamate, 4-(3-dimethylaminopropyl)-piperazine carbamate, and

(iv) when R is aziridino or ethoxy, R^1 is hydroxymethyl or a carbamate or

30 C_{1-6} aliphatic acyl ester thereof; R^4 is methyl or bromo and R^3 is ethyl, R^2 is not methyl.

23. A compound as claimed in Claim 22 characterised in that R^1 is hydroxymethyl or a group $-CH_2-X$.
24. A compound as claimed in Claim 22 or 23 characterised in that R is aziridin-1-yl or 2-methylaziridin-1-yl.
25. A compound as claimed in Claim 22, 23 or 24 characterised in that R^3 is C_{1-4} alkyl or haloalkyl.
26. A compound as claimed in Claim 22, 23, 24 or 25 characterised in that R^2 is $n-C_{1-4}$ alkyl, isopropyl, or C_{3-7} cycloalkyl.
27. A compound as claimed in claim 22 and as described herein as one of compound numbers 21, 22, 24, 25, 26, 37, 40, 46, 53, 54, 55, 63, 64, 65, 68, 69, 70, 71, 72, 73, 75, 80, 81, 82 or 84.
28. A process for preparing an optionally substituted aziridin-1-yl compound of formula II as described herein wherein R^1 is alkoxycarbonyl characterised in that it comprises reacting a corresponding 2-substituted -3-alkoxycarbonyl-5-alkoxy-1-alkylindole-4,7-dione compound with an optionally substituted aziridine.
29. A process for preparing a compound of formula Ia as described herein wherein R^1 is a hydroxymethyl group characterised in that it comprises reacting a corresponding 2-substituted-3-alkoxy-1-alkylindole-4,7-dione with an oxidising reagent and then reducing the resultant hydroquinone.

30. A compound of general formula II



characterised in that R is C₁₋₄ alkoxy, R¹ is C₁₋₄ alkoxycarbonyl and R², R³ and R⁴ are as described in any one of claims 22 to 26.

31. A method of treating a human or animal body for the purposes of preventing growth of, and/or killing, neoplasm cells comprising administering a compound or
5 pharmaceutically acceptable salt thereof as claimed for use in any one of claims 1 to 21 or as claimed *per se* in any one of claims 22 to 26 to the body in a dose sufficient to prevent growth of or kill some or all of the neoplasm cells.

32. A method as claimed in claim 31 wherein the cells are tumour cells and/or cancer cells.

10 33. A method as claimed in claim 32 wherein the cells are hypoxic and/or anoxic cancer cells.

34. A method as claimed in any one of claims 31 to 33 comprising administering the compound or salt to the body within a set period of treatment of the body with doses of radiation, the doses of radiation being sufficient to prevent growth of or kill some or all
15 of the cells supplied with blood.

35. A method as claimed in any one of claims 31 to 34 wherein the compound and radiation are administered within 1 to 30 days of each other.

36. A method as claimed in claim 35 wherein they are administered on the same day or within 7 days of each other.

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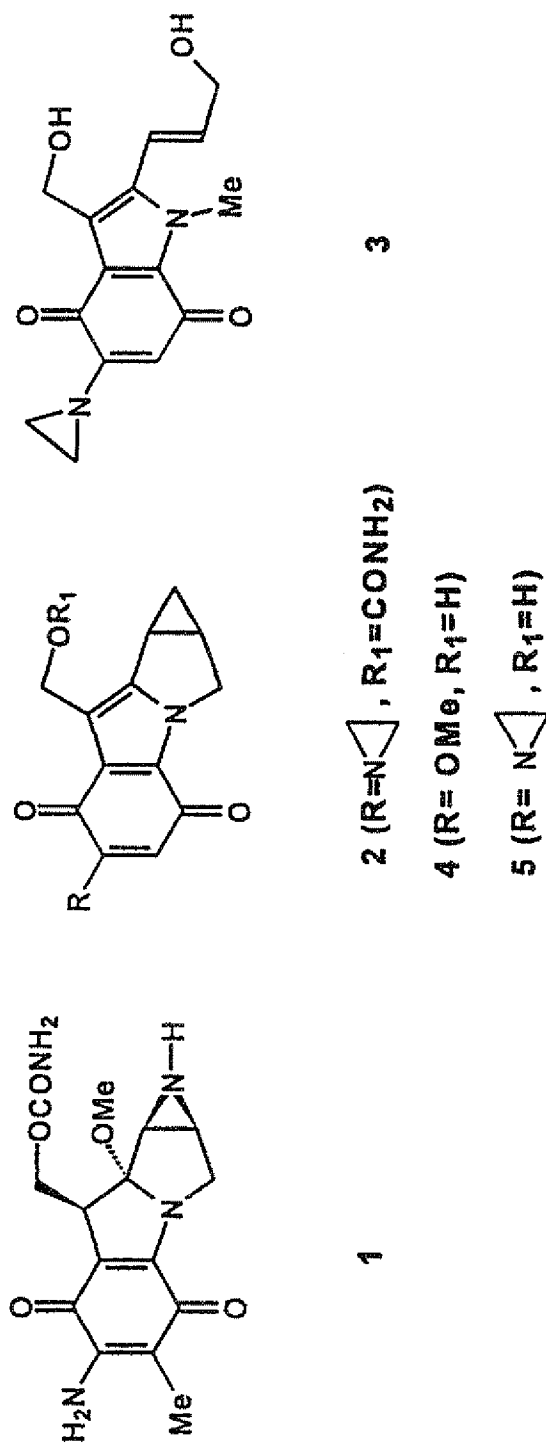


Fig. 1

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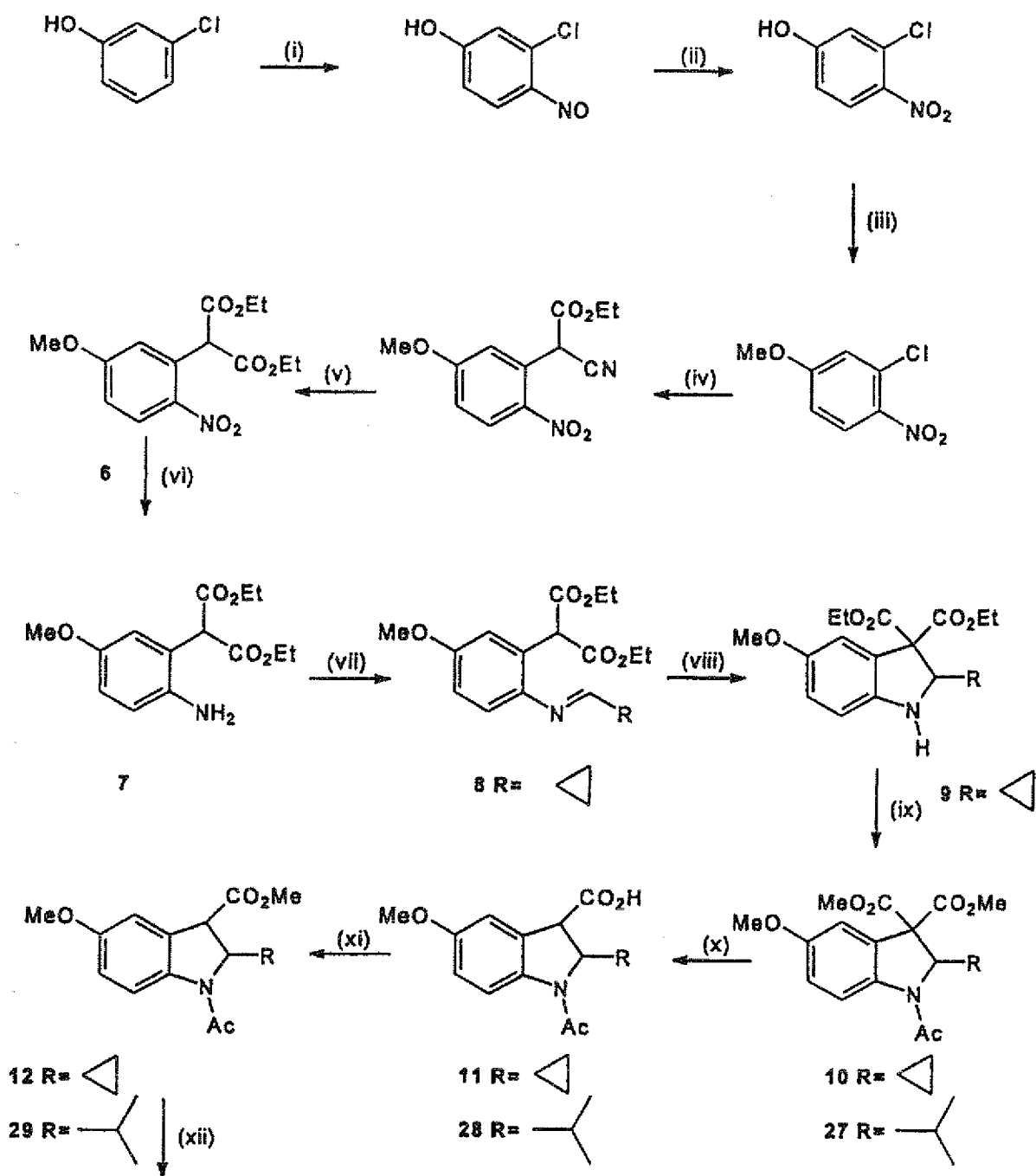


Fig. 2

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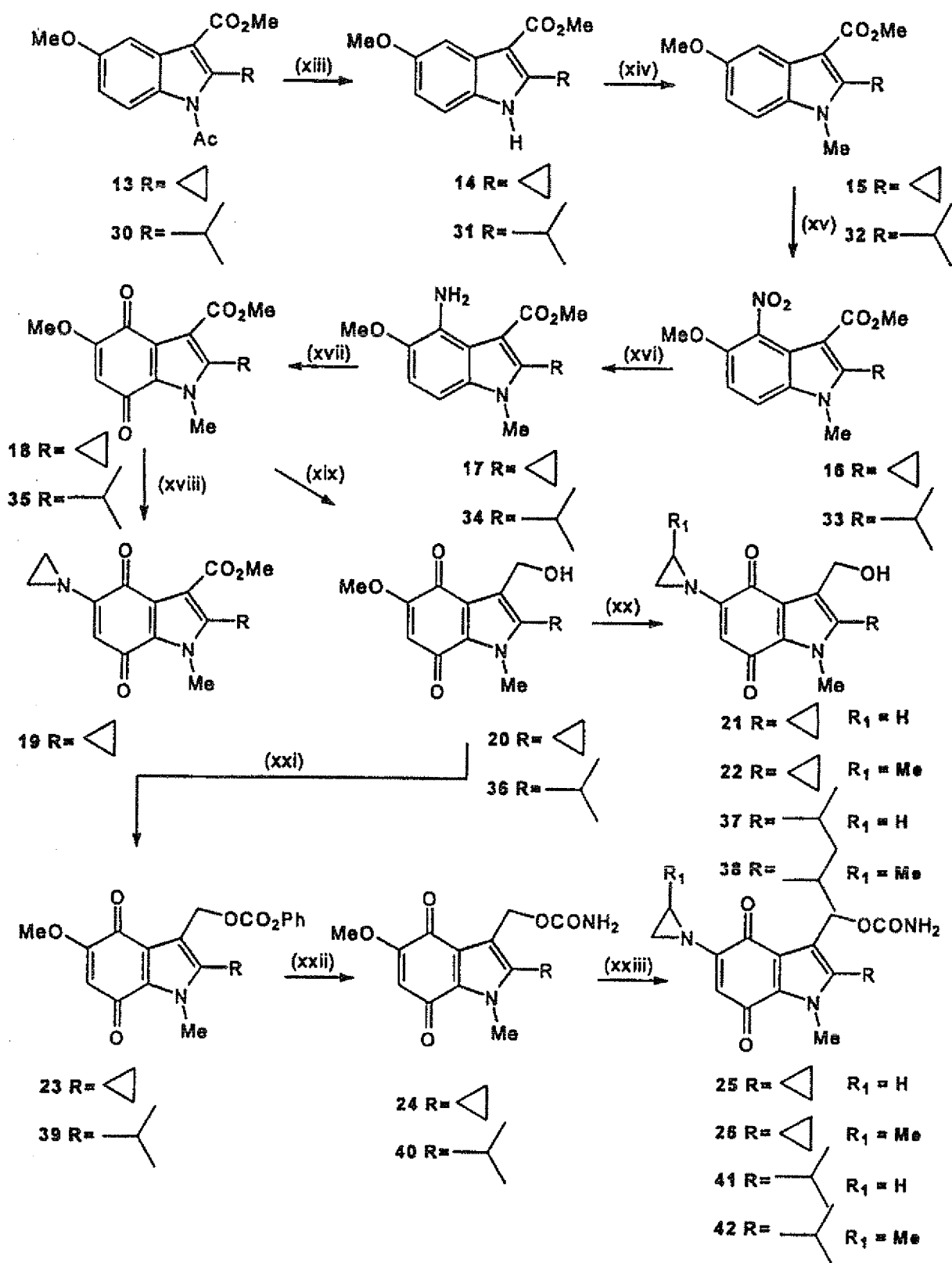


Fig. 3

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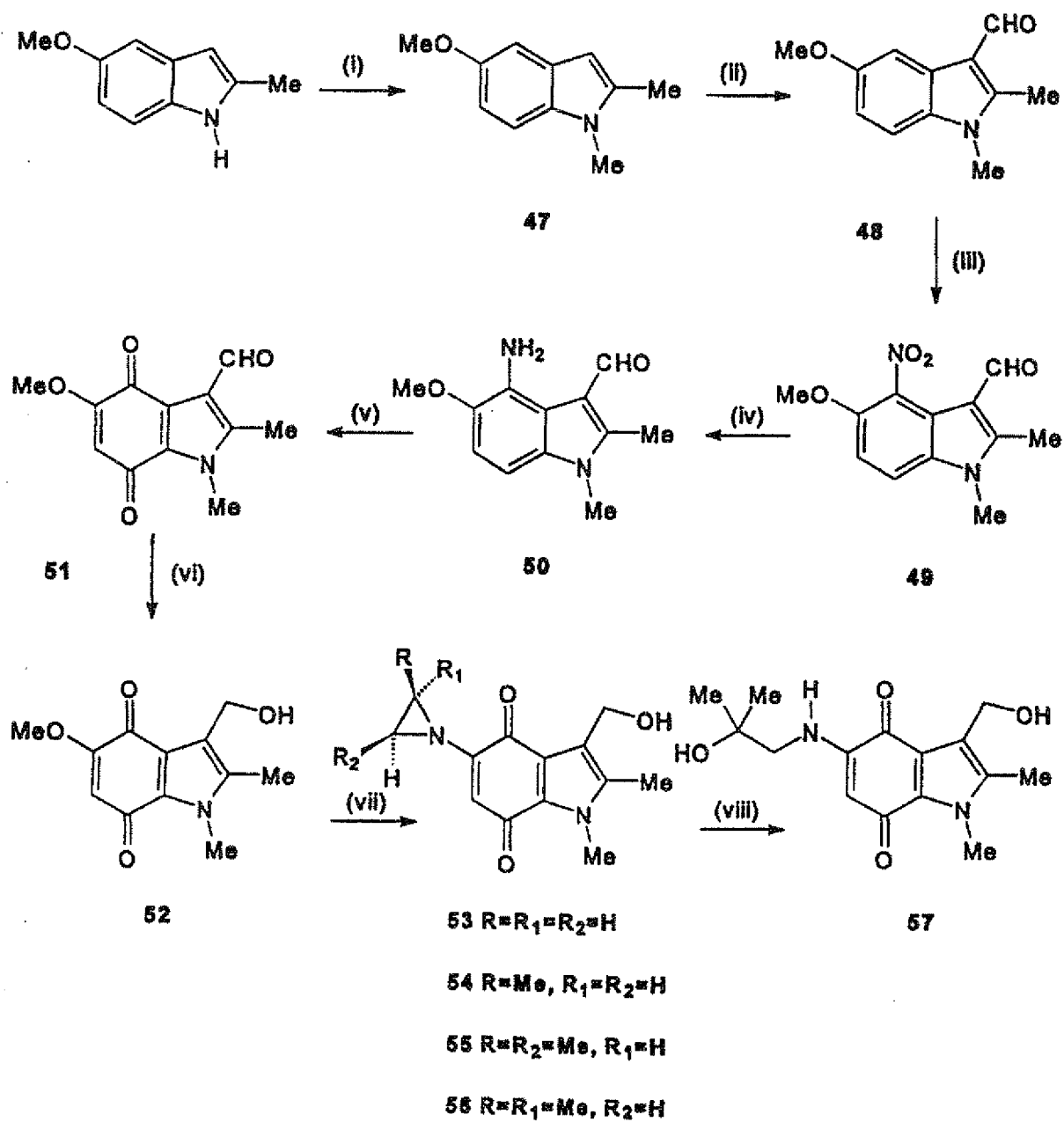


Fig. 4

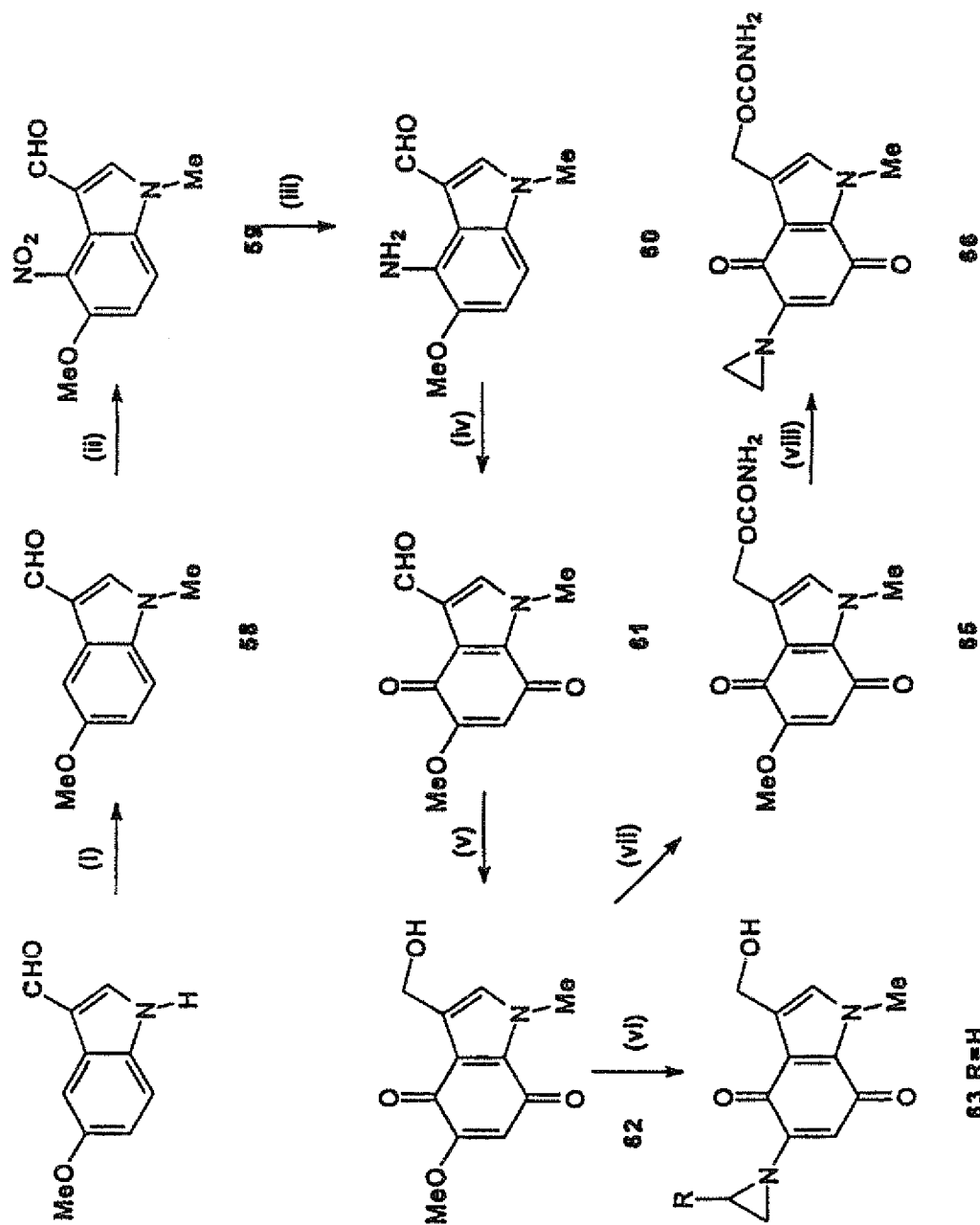


Fig.5

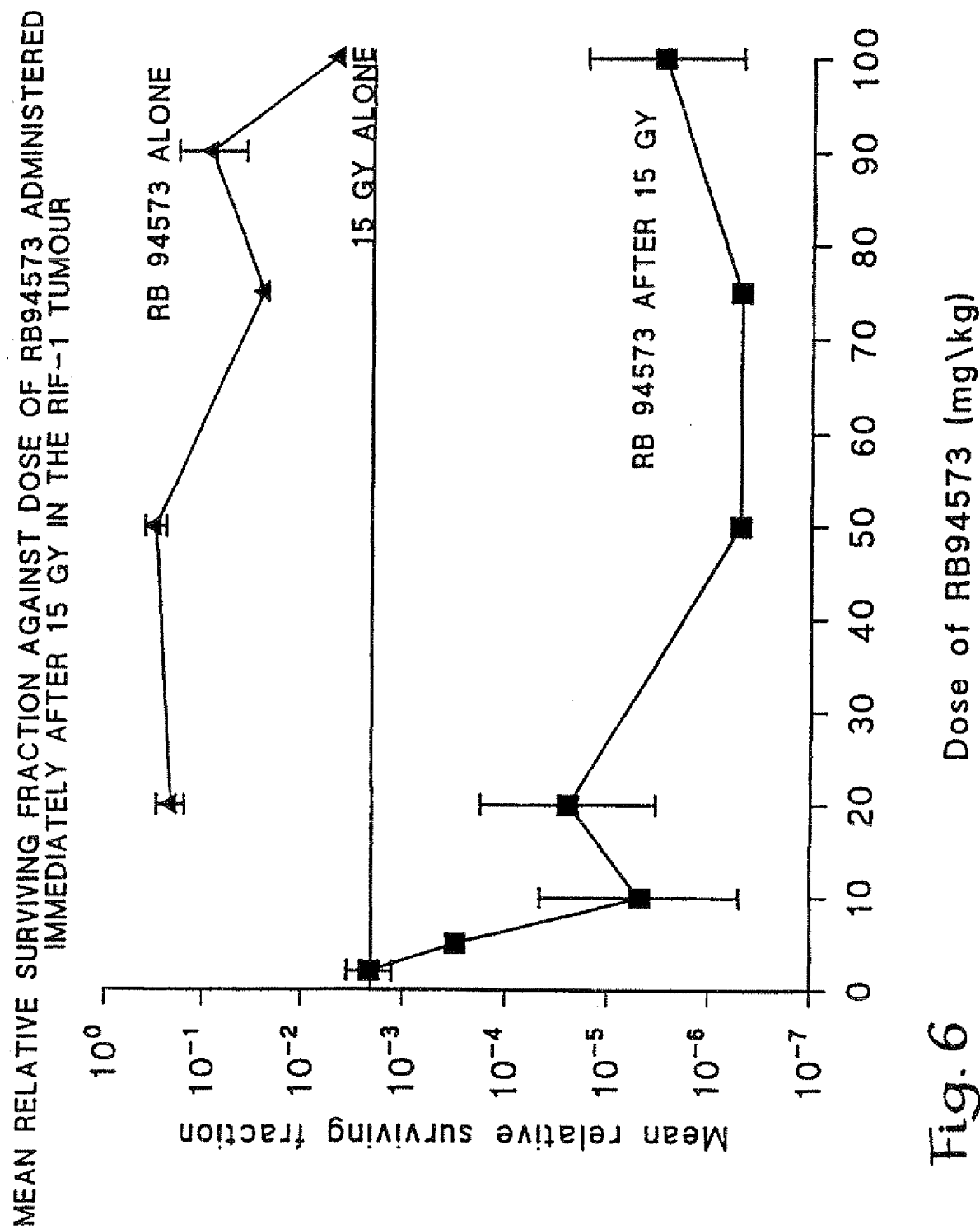


Fig. 6

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MEAN RELATIVE SURVIVING FRACTION AGAINST DOSE OF RB94577 ADMINISTERED IMMEDIATELY AFTER 15 GY IN THE RIF-1 TUMOUR

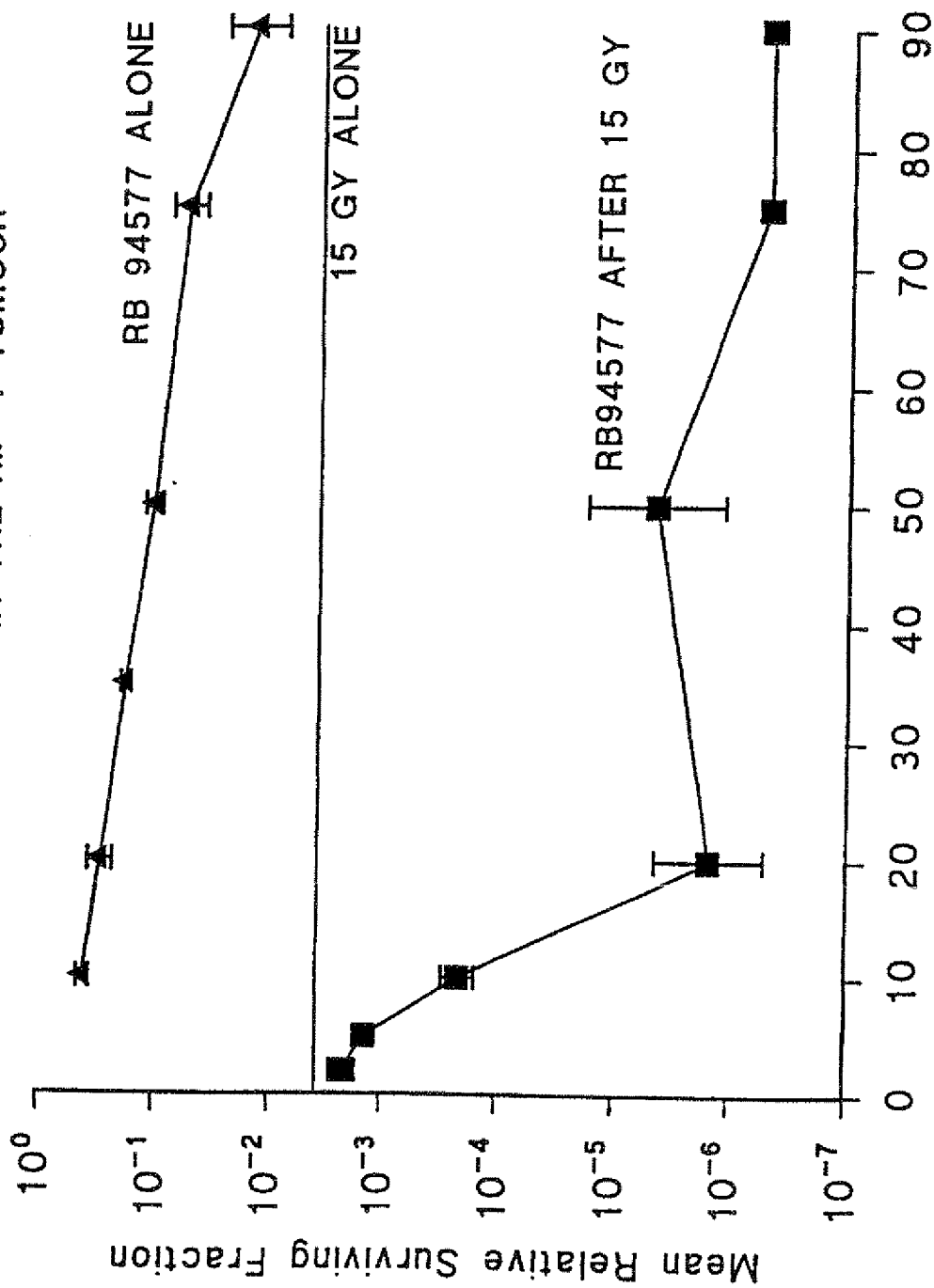


Fig. 7

MEAN RELATIVE SURVIVING FRACTION AGAINST DOSE OF RB94573 ADMINISTERED IMMEDIATELY AFTER 10 GY IN THE KHT TUMOUR

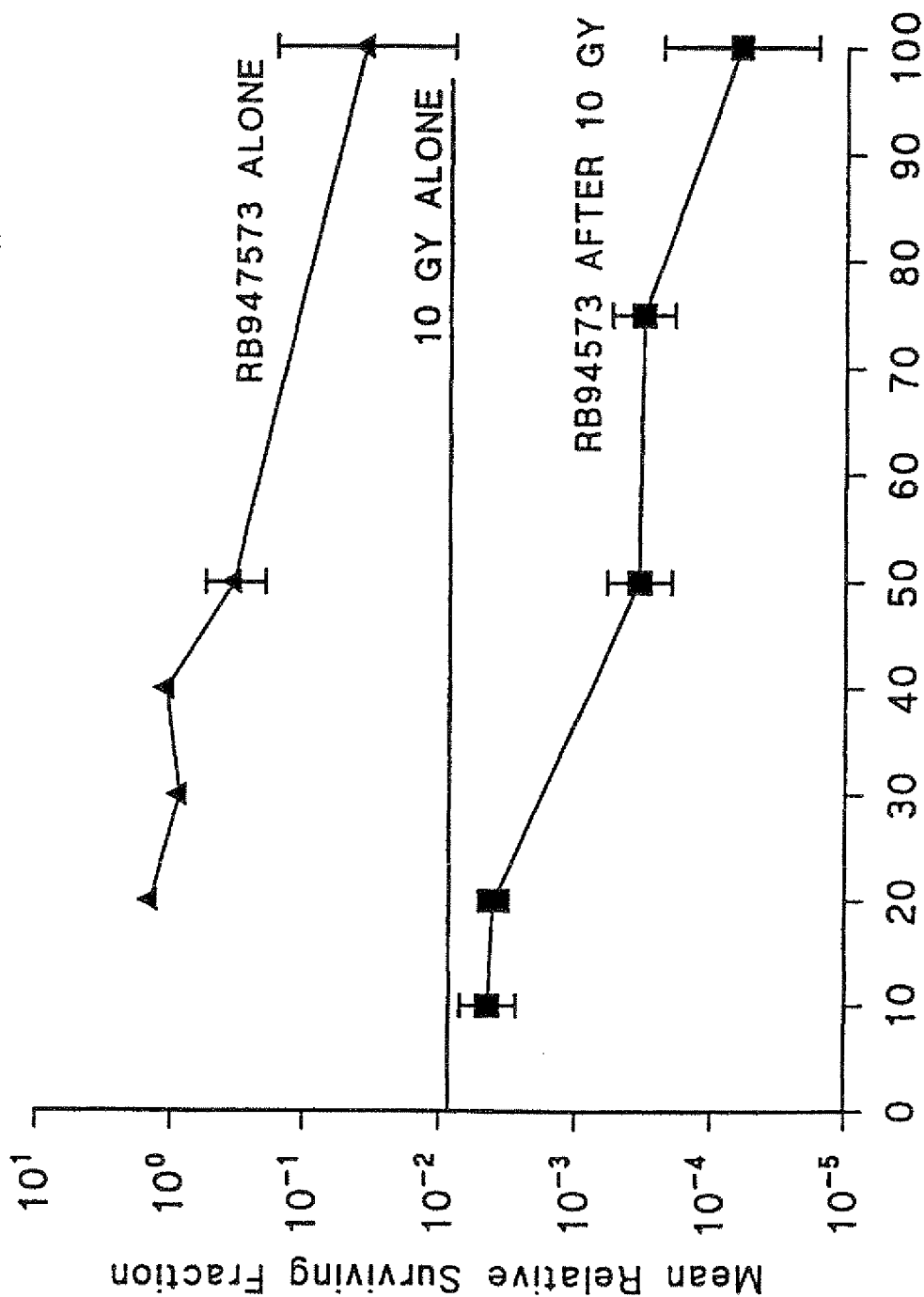


Fig.8 Dose of RB94573 (mg/kg)

MEAN RELATIVE SURVIVING FRACTION AGAINST DOSE OF RB94577 ADMINISTERED IMMEDIATELY AFTER 10 GY IN THE KHT TUMOUR

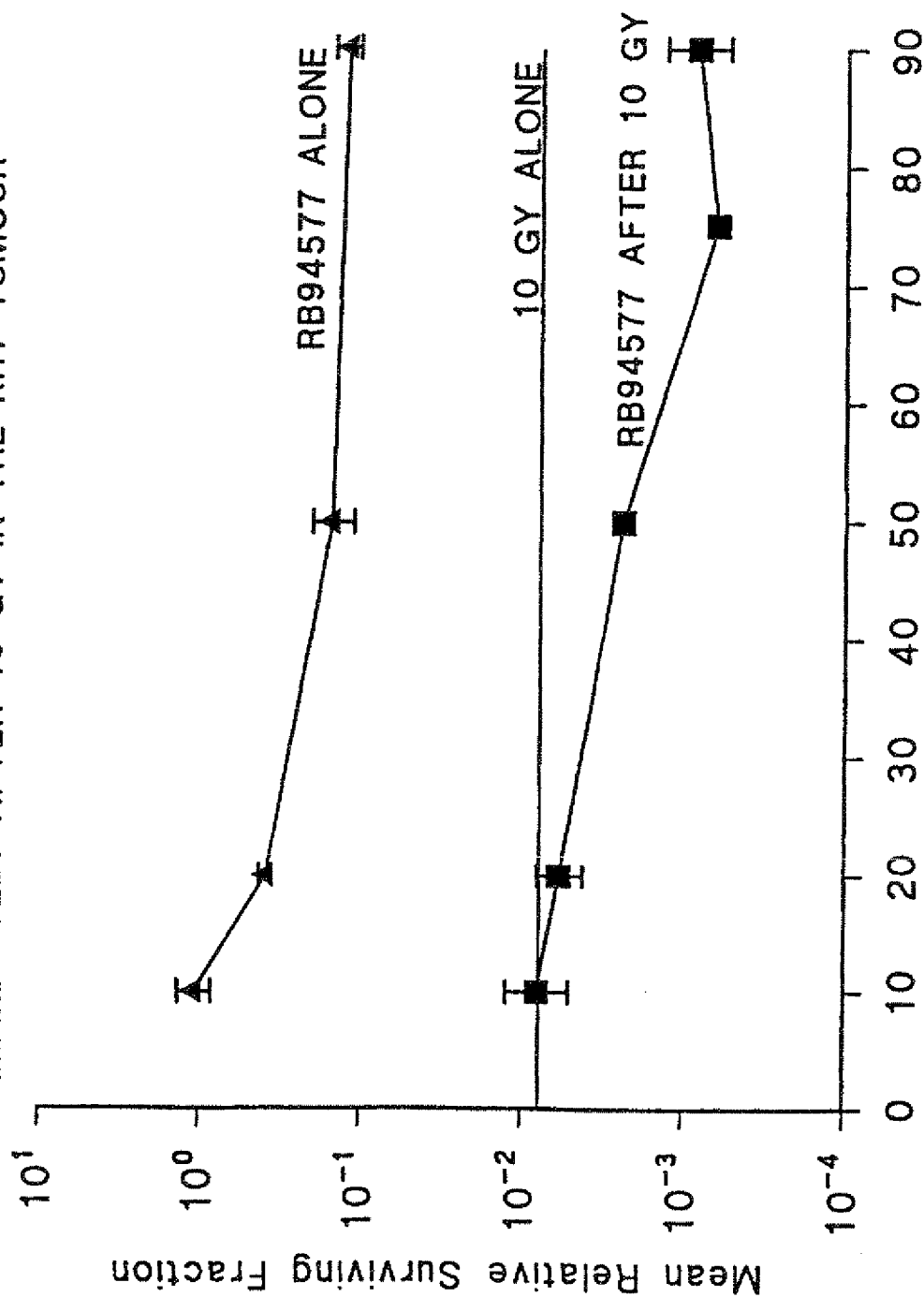


Fig. 9 Dose of RB 94577 (mg/kg)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/03176

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D209/42 A61K31/40 C07D209/12 C07D403/04
/(C07D403/04,209:00,203:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>JOURNAL OF MEDICINAL CHEMISTRY, vol. 37, no. 22, 1994, pages 3834-3843, XP002027789 A.S. COTTERILL ET AL.: "Cyclopropamitosenes, Novel Bioreductive Anticancer Agents. Synthesis, Electrochemistry, and Biological Activity of 7-Substituted Cyclopropamitosenes and Related Indolequinones" cited in the application * See the whole document, in particular Table 4, p. 3837 *</p> <p style="text-align: center;">--- -/--</p>	1-30

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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